

# Construction of Pbs.PGK.PCR1

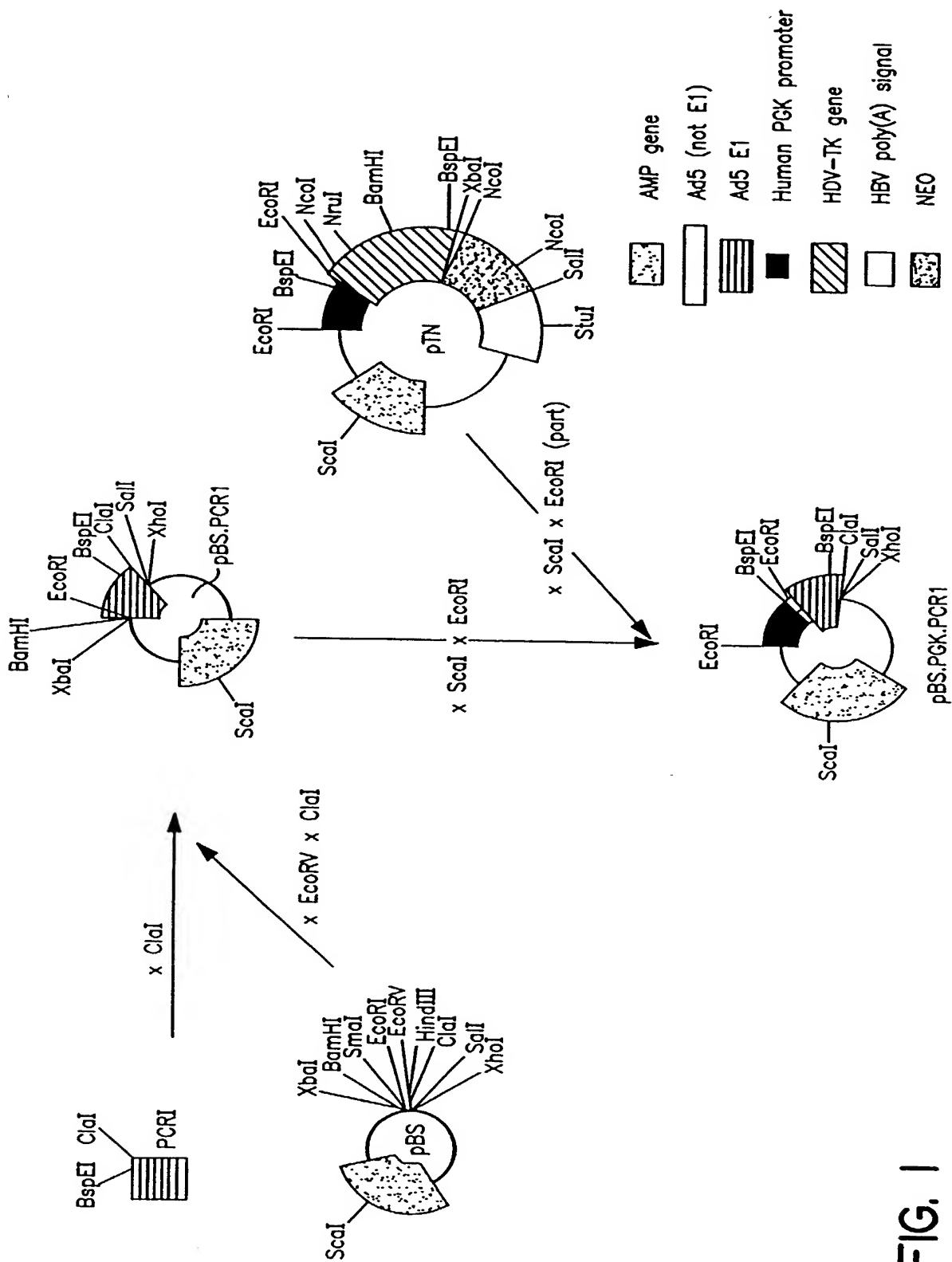


FIG. 1



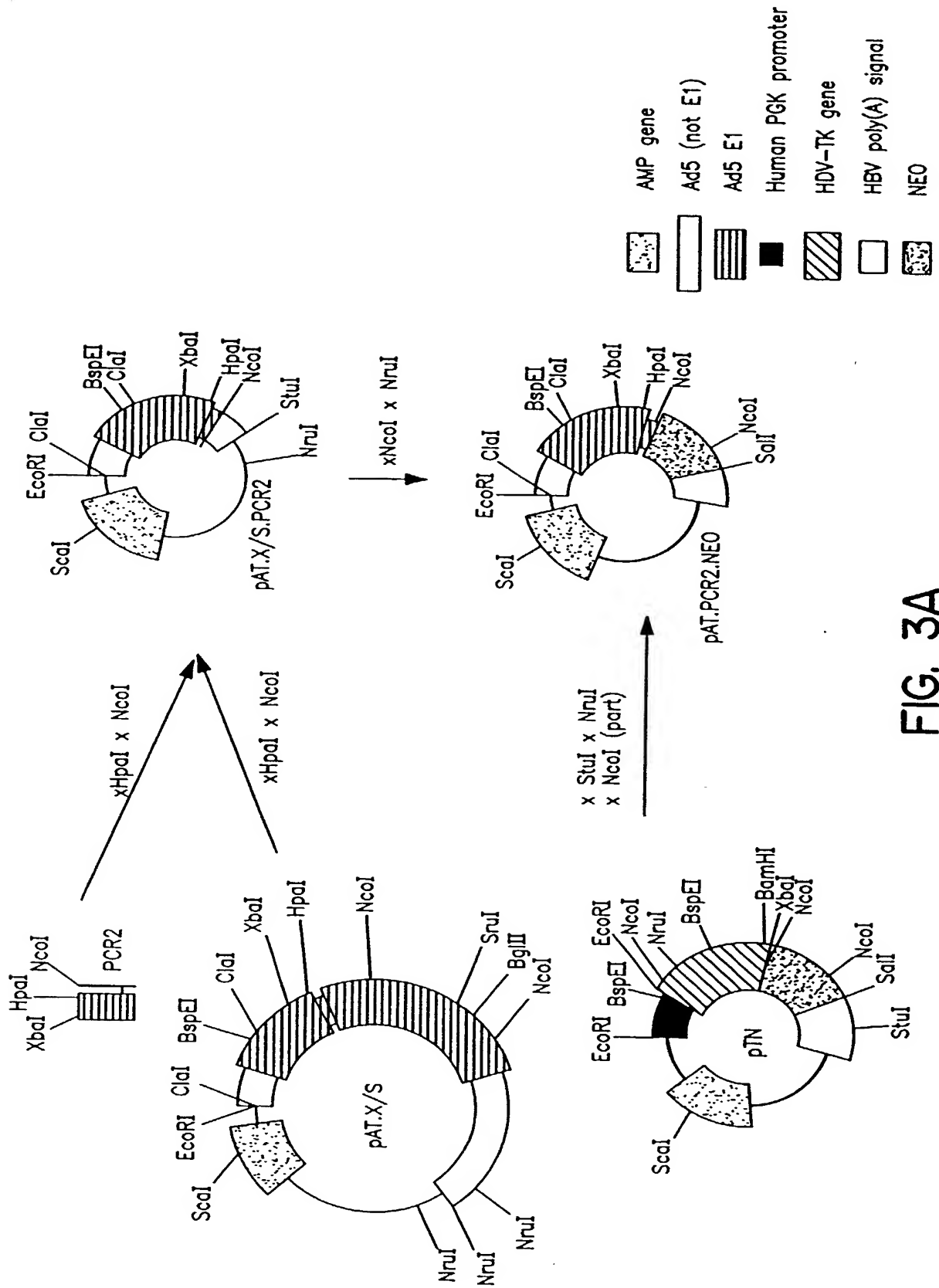


FIG. 3A

# Construction of pIG.E1a.NEO

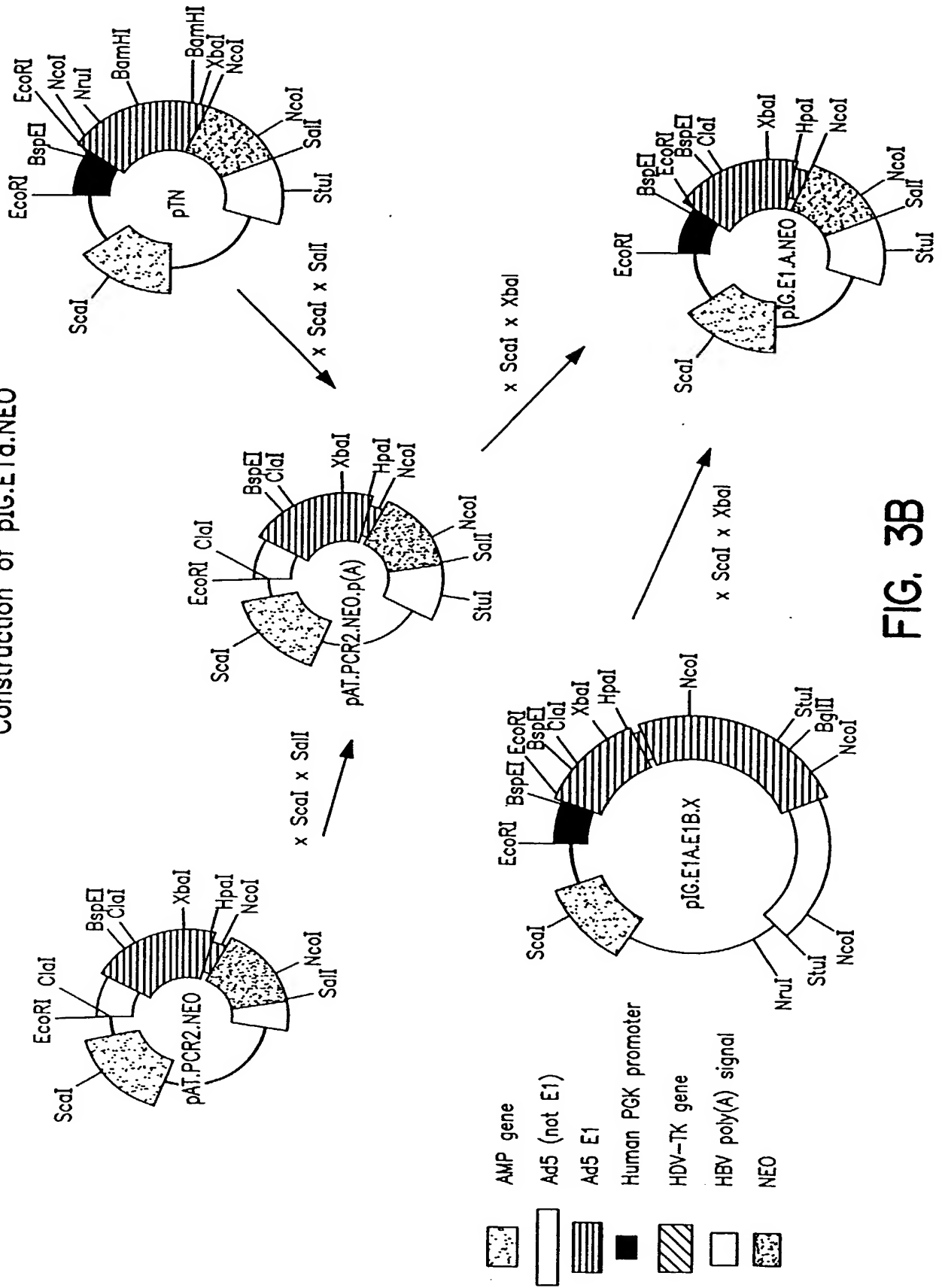
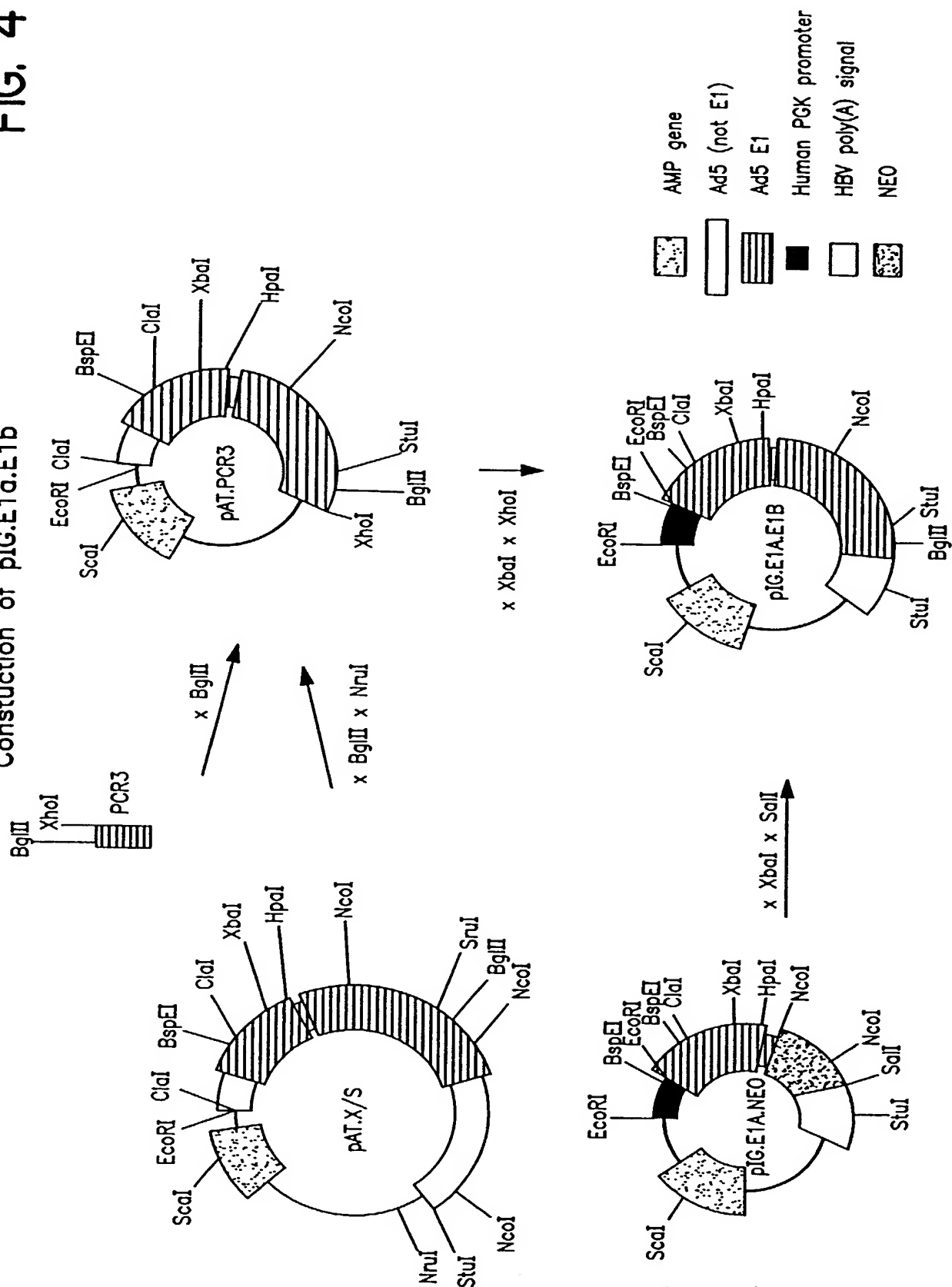


FIG. 3B

FIG. 4

Construction of pIG.E1a.E1b



# Construction of pIG.NEO

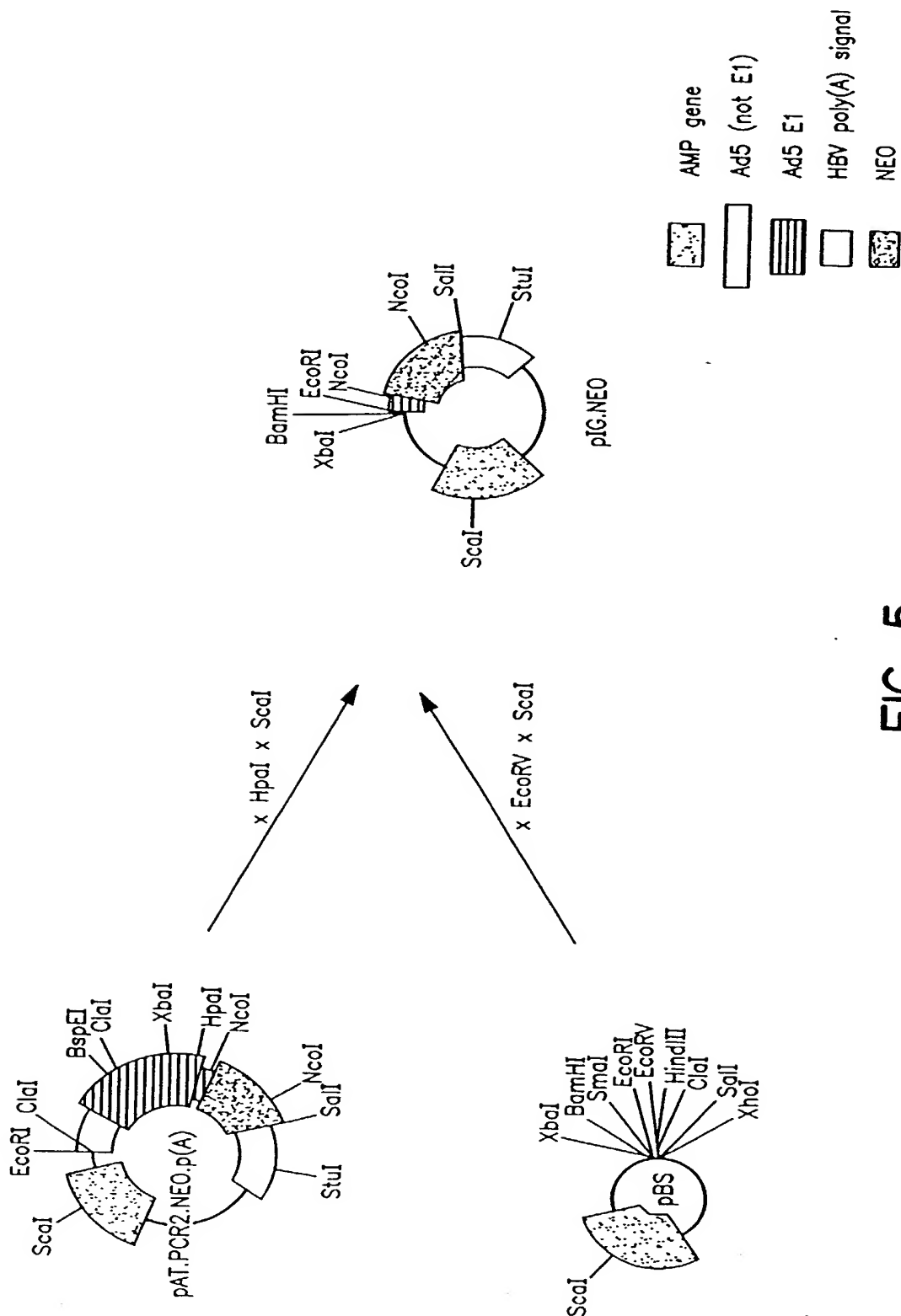


FIG. 5

# Overview of available adenovirus packaging constructs and assessment of their capacity to transform primary kidney cells

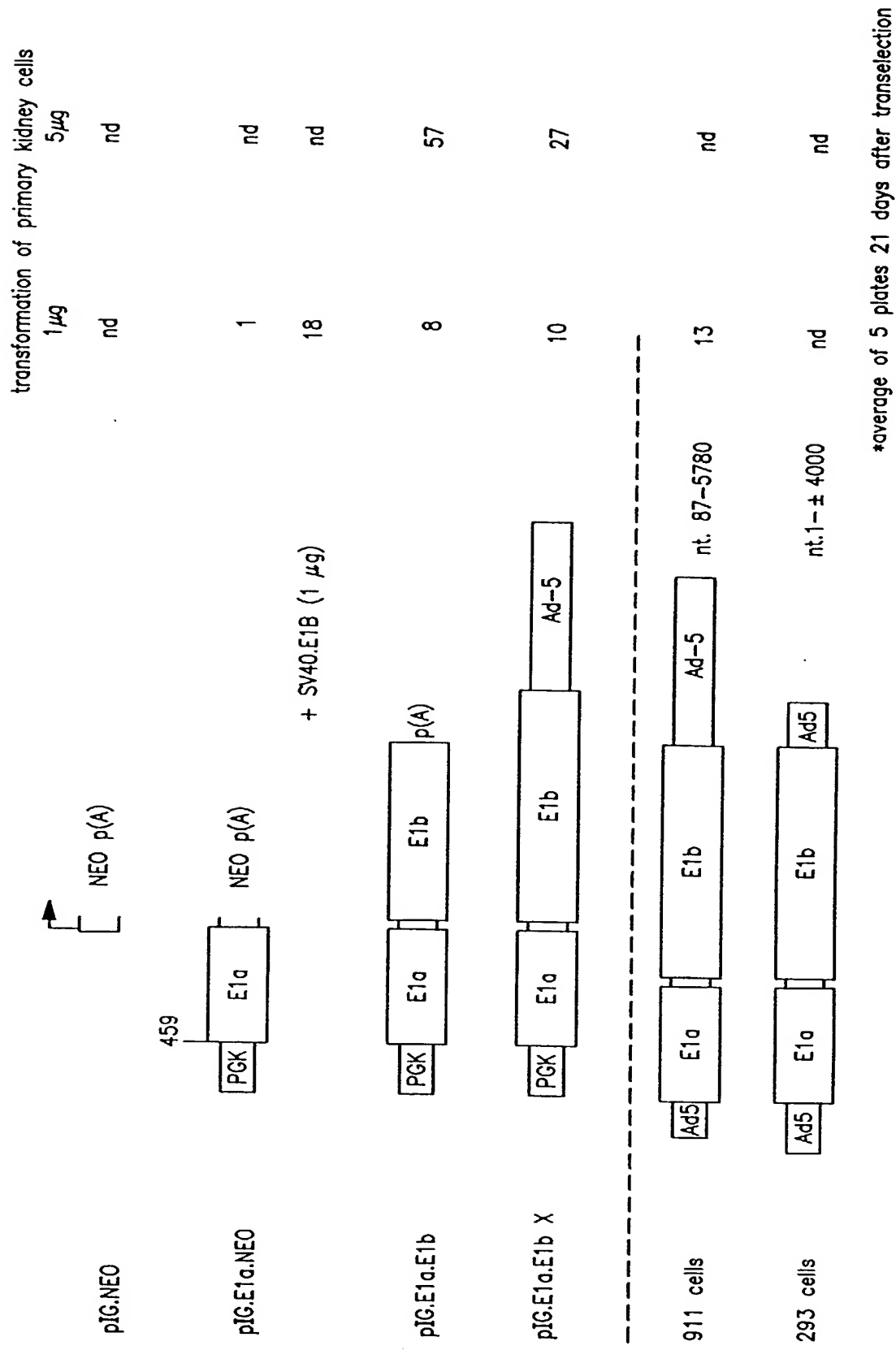


FIG. 6

Western blotting analysis of A549 clones transfected with pIG.E1A.NEO and PER clones (HER cells transfected with pIG.E1A.E1B)

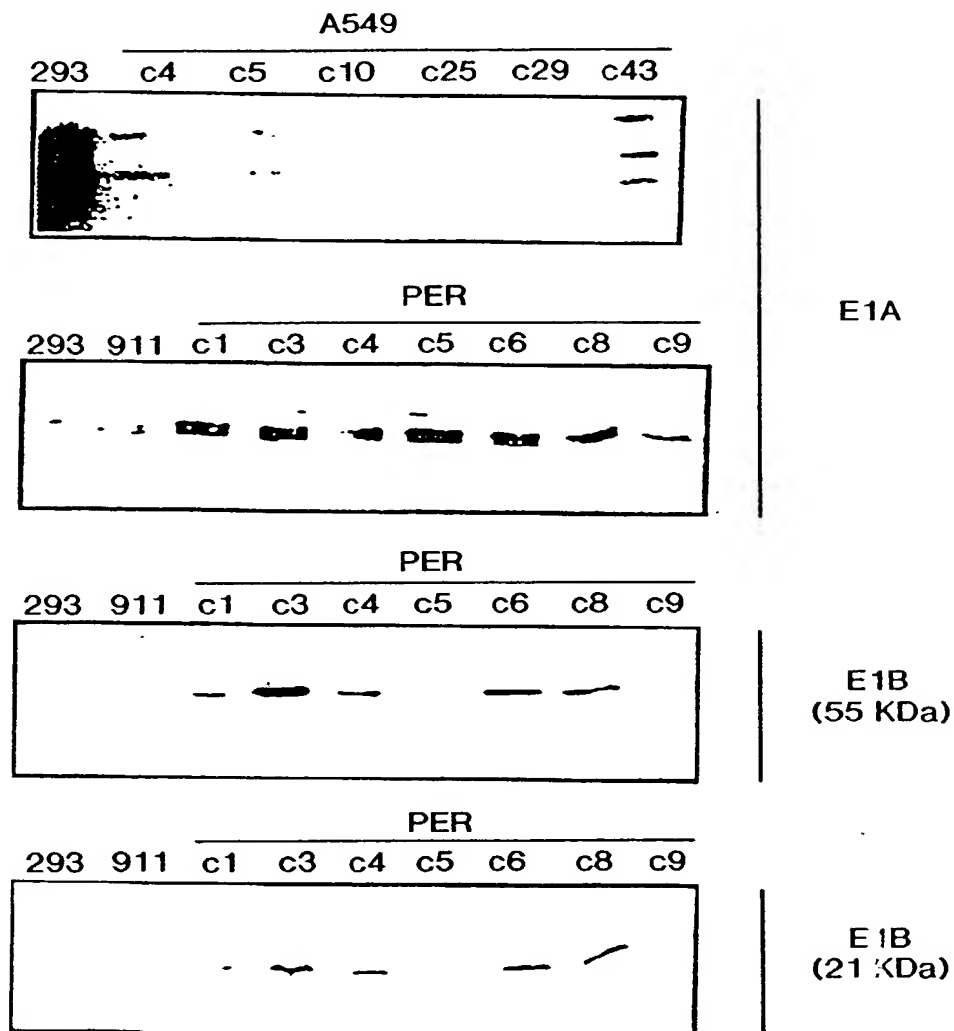


FIG. 7



## Southern blot analyses of 293, 911 and PER cell lines

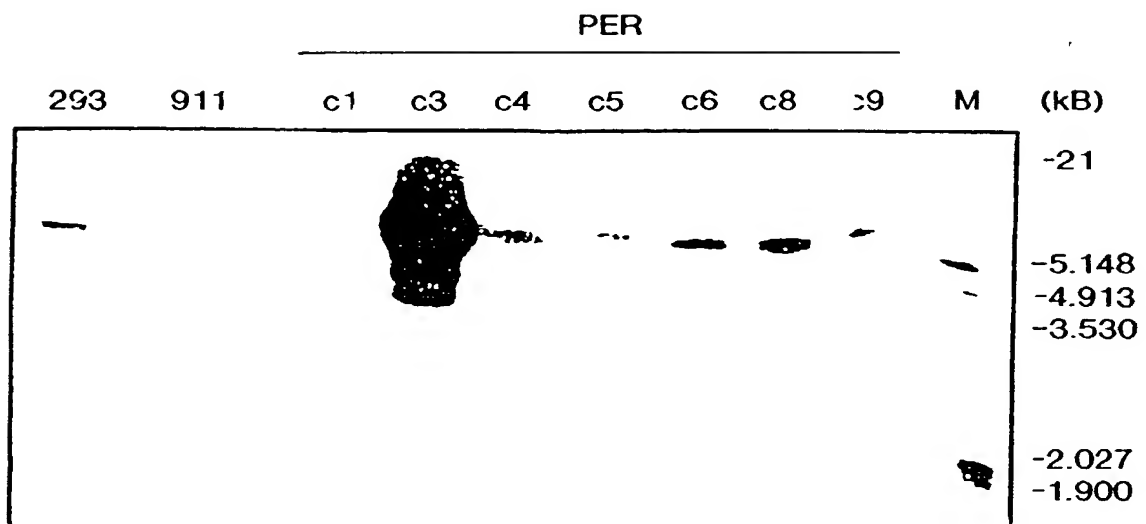


FIG. 8

Transfection efficiency of PER.C3, PER.C5, PER.C6 and 911 cells. Cells were cultured in 6-well plates and transfected (n=2) with 5  $\mu$ g pRSV.lacZ by calcium-phosphate co-precipitation. Forty-eight hours later the cells were stained with X-GAL. The mean percentage of blue cells is shown.

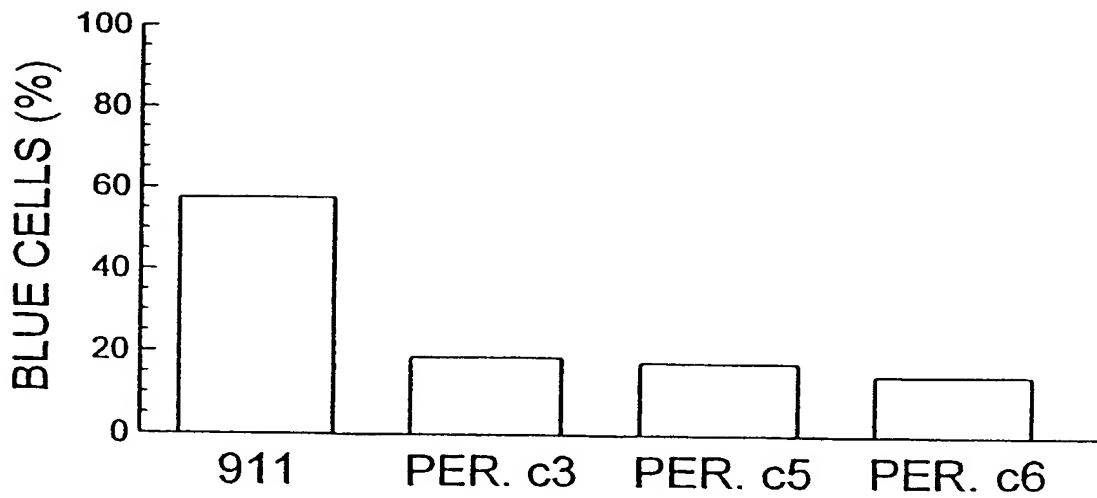


FIG. 9

# Construction of pMLP1.TK

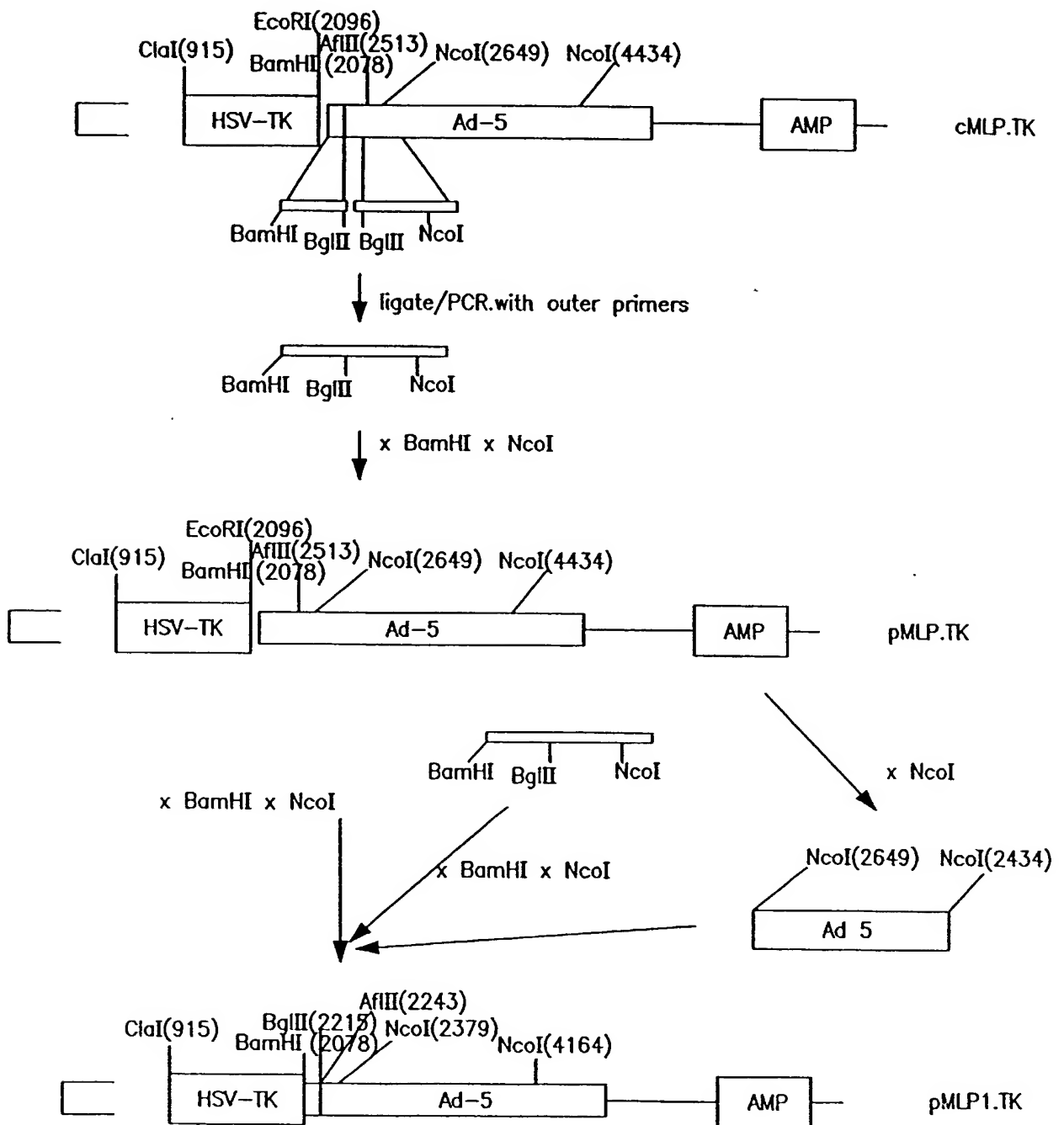


FIG. 10

# New recombinant adenoviruses and packaging constructs without sequence overlap

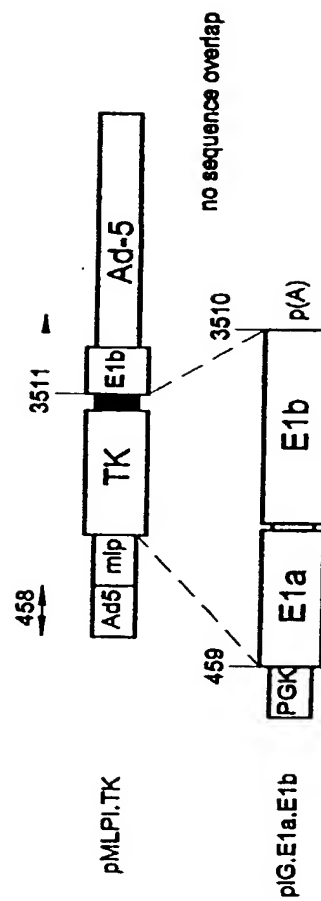
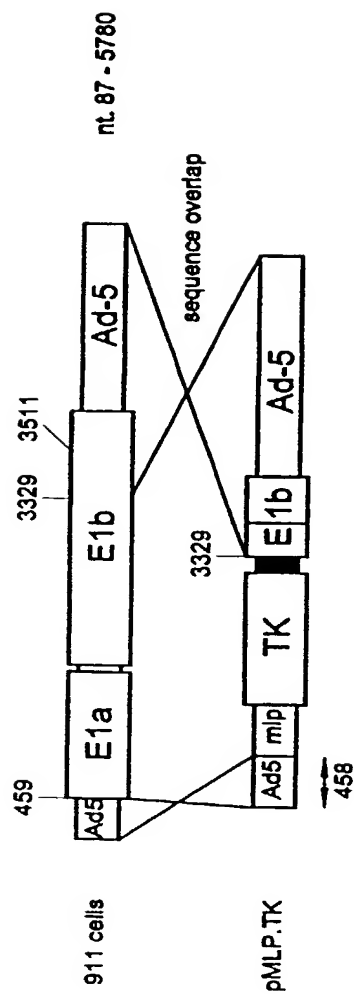
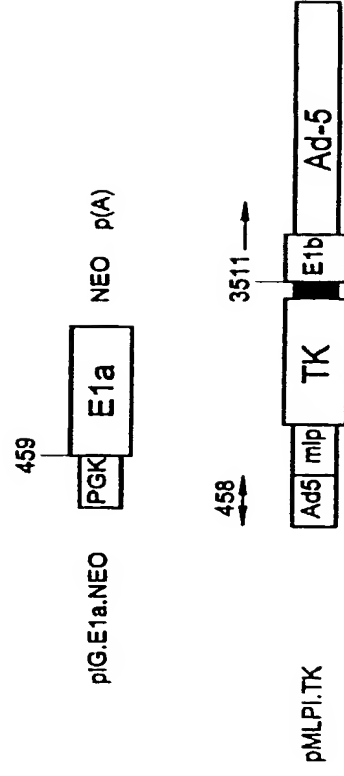


FIG. 1IA

Packaging system based on primary cells

1993/05/19 10:00:00

New recombinant adenoviruses and packaging constructs without sequence overlap



Packaging system based on established cell lines: transfection with E1a and selection with G418 **FIG. 1B**

# Generation of recombinant adenovirus

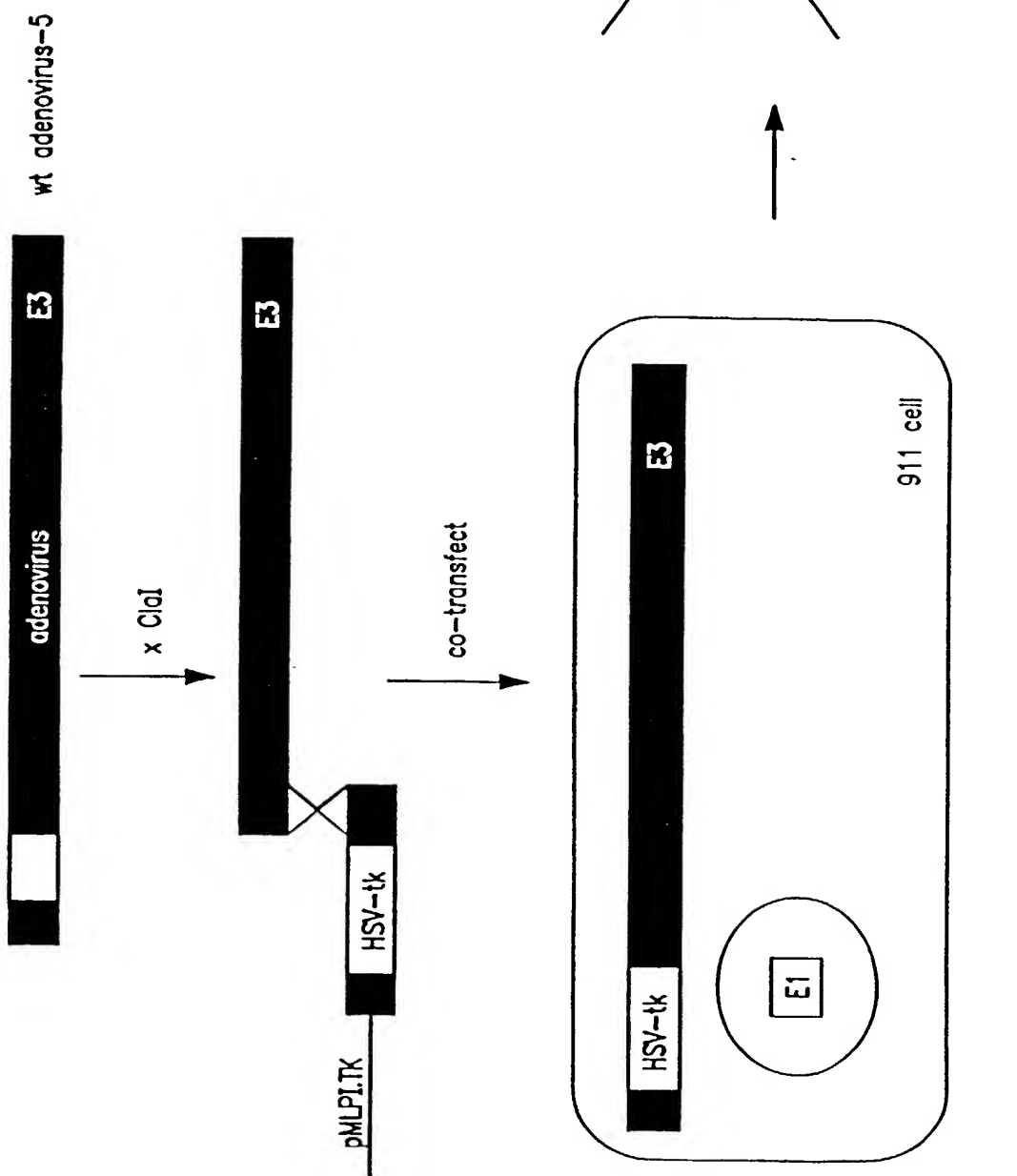


FIG. 12

FIG. 13

# Replication of Adenovirus

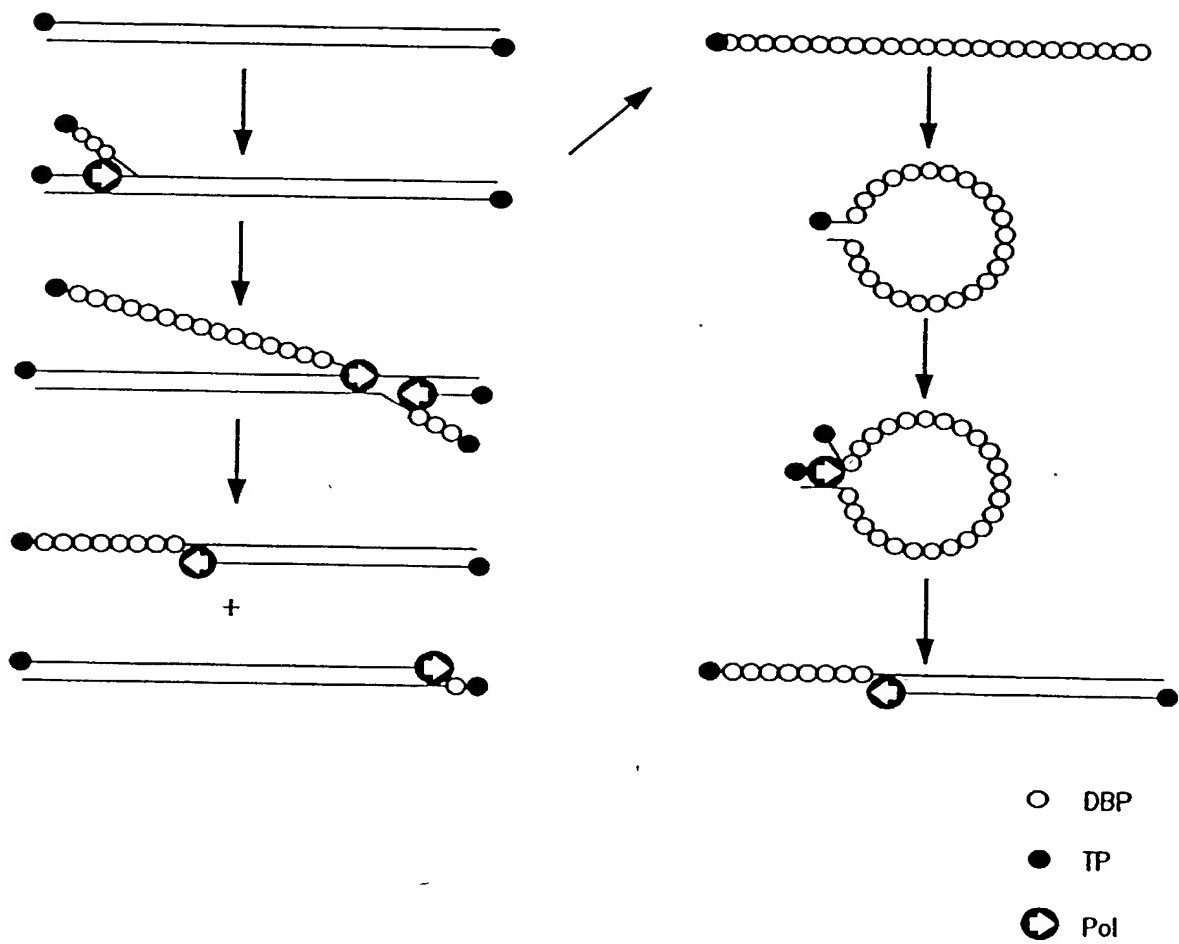


FIG. 14



The potential hairpin conformation of a single-stranded DNA molecule that contains the HP/asp sequences used in these studies. Restriction with the restriction endonucleases *Asp718I* of plasmid pICLHa, containing the annealed oligonucleotide pair HP/asp1 en HP/asp2 will yield a linear double-stranded DNA fragment. In cells in which the required adenovirus genes are present, replication can initiate at the terminus that contains the ITR sequence. During the chain elongation, the one of the strands will be displaced. The terminus of the single-stranded displaced-strand molecule can adopt the conformation depicted above. In this conformation the free 3'-terminus can serve as a primer for the cellular and/or adenovirus DNA polymerase, resulting in conversion of the displaced strand in a double-stranded form.

```

5'-GTAACTGACCTAGTGCCGCCCCGGGCA
      |||||
3'-GATCACGGCGGGCCCCGA
  
```

FIG. 15

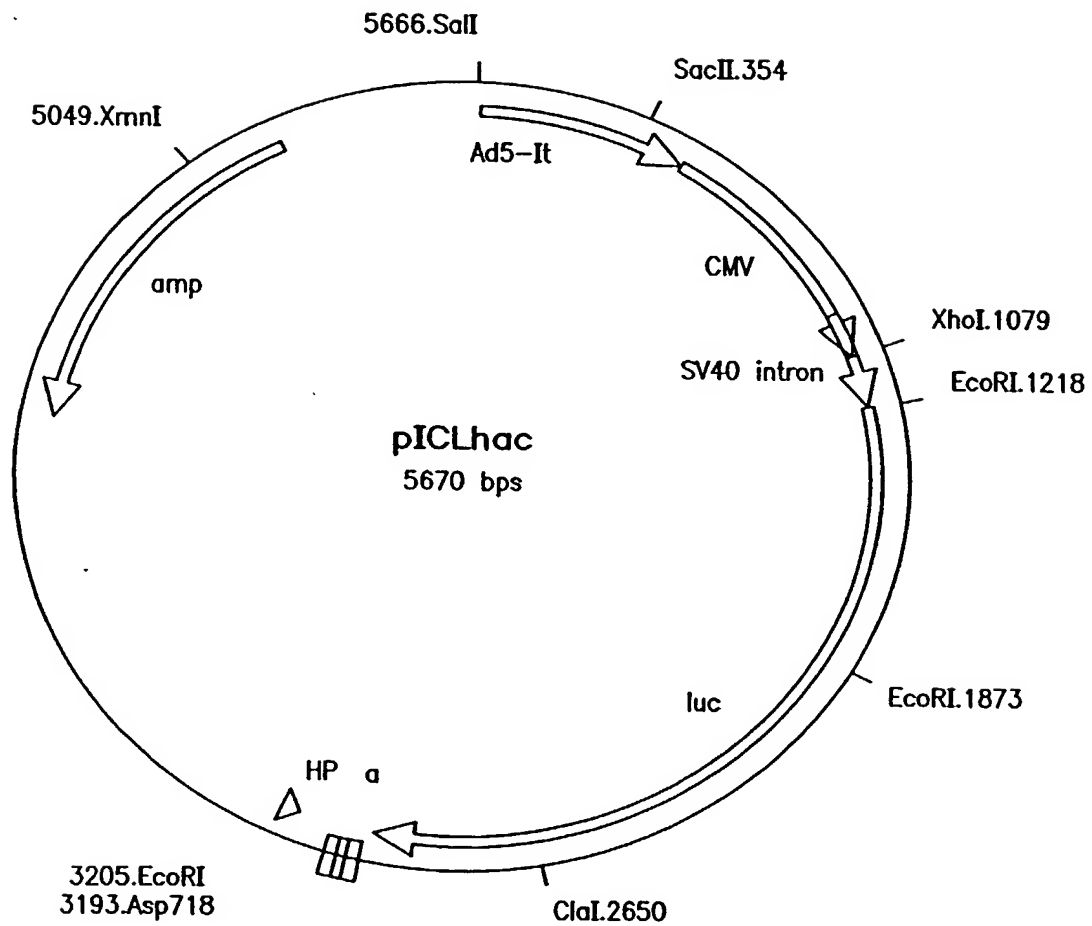


FIG. 16

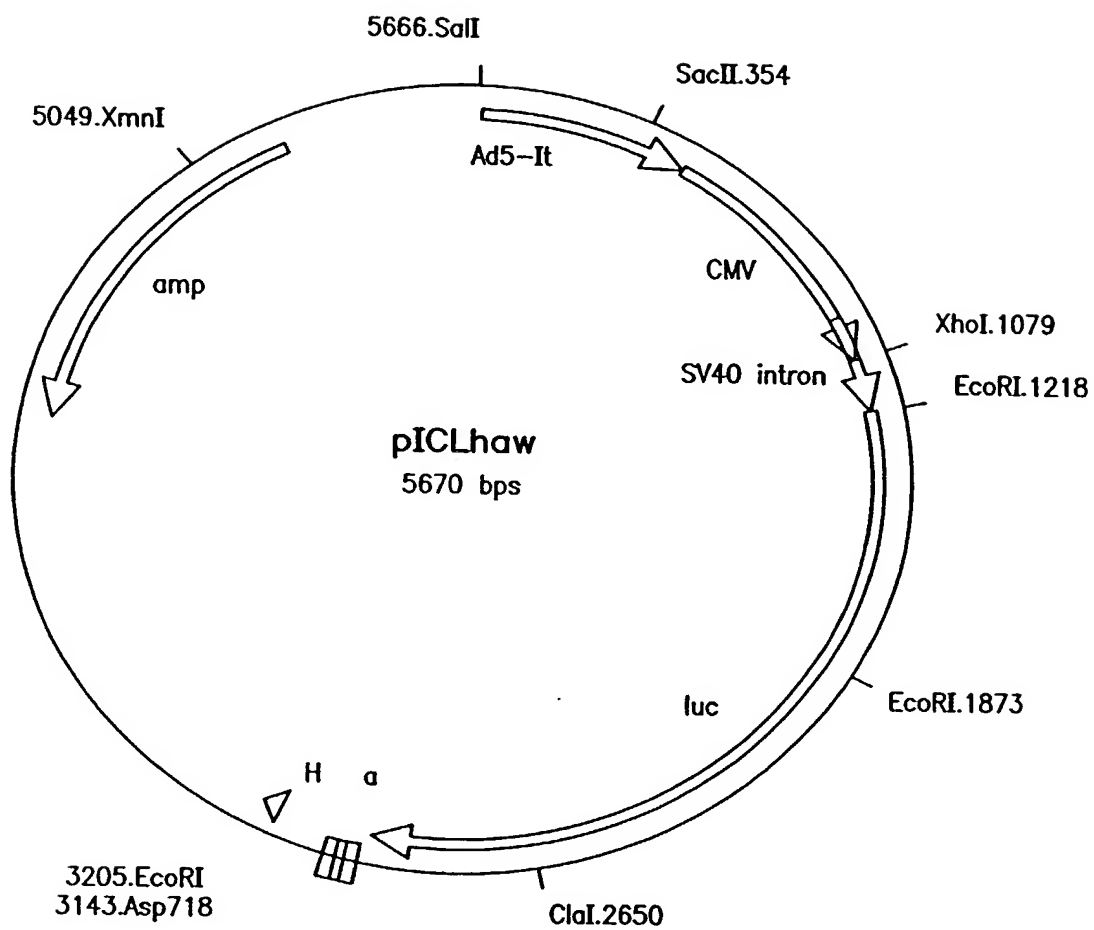


FIG. 17

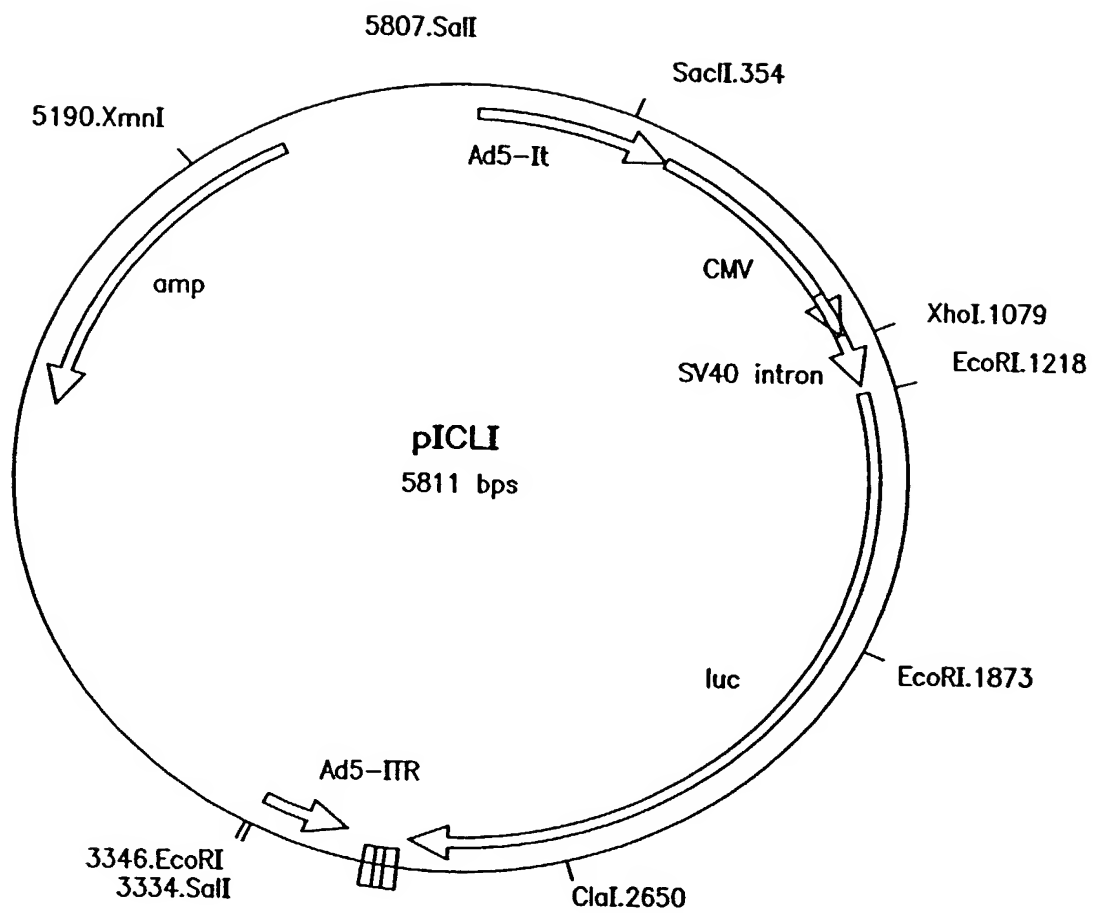


FIG. 18

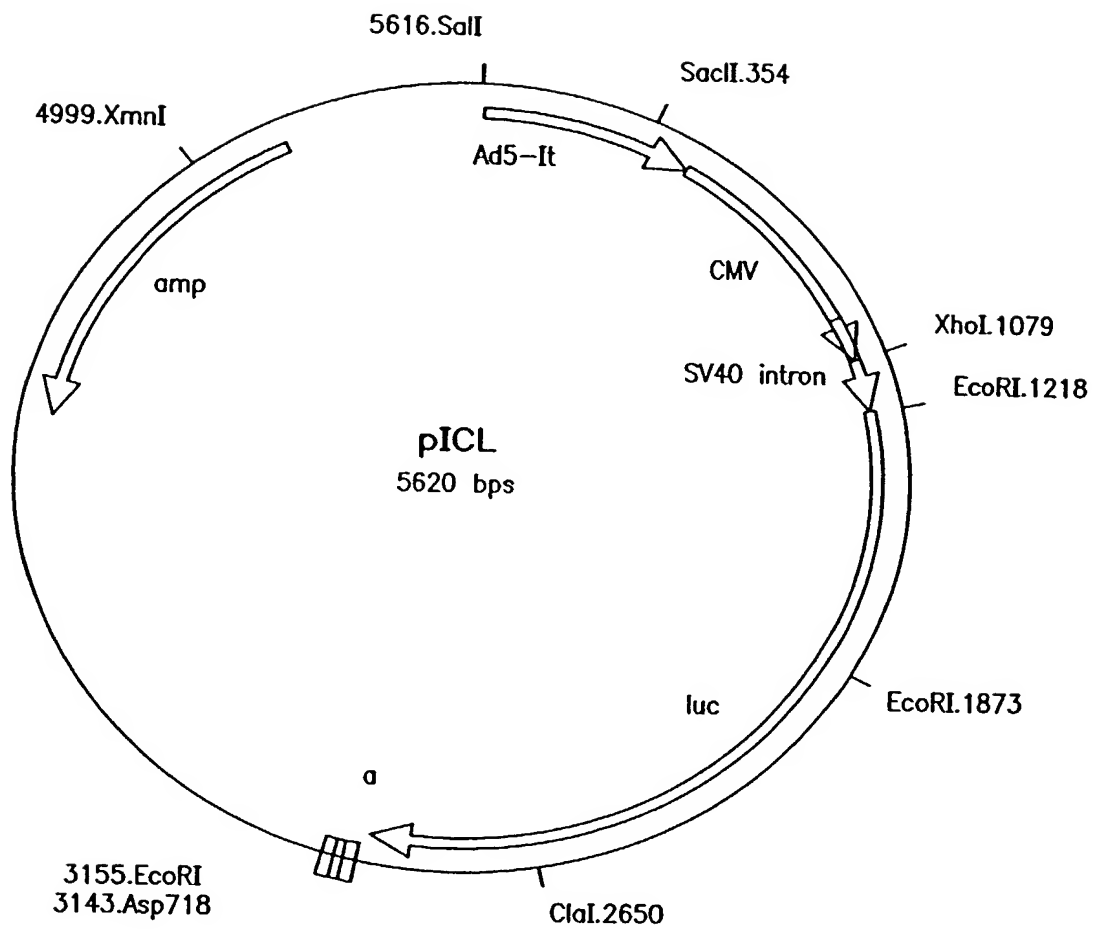


FIG. 19

# Cloned adenovirous fragments

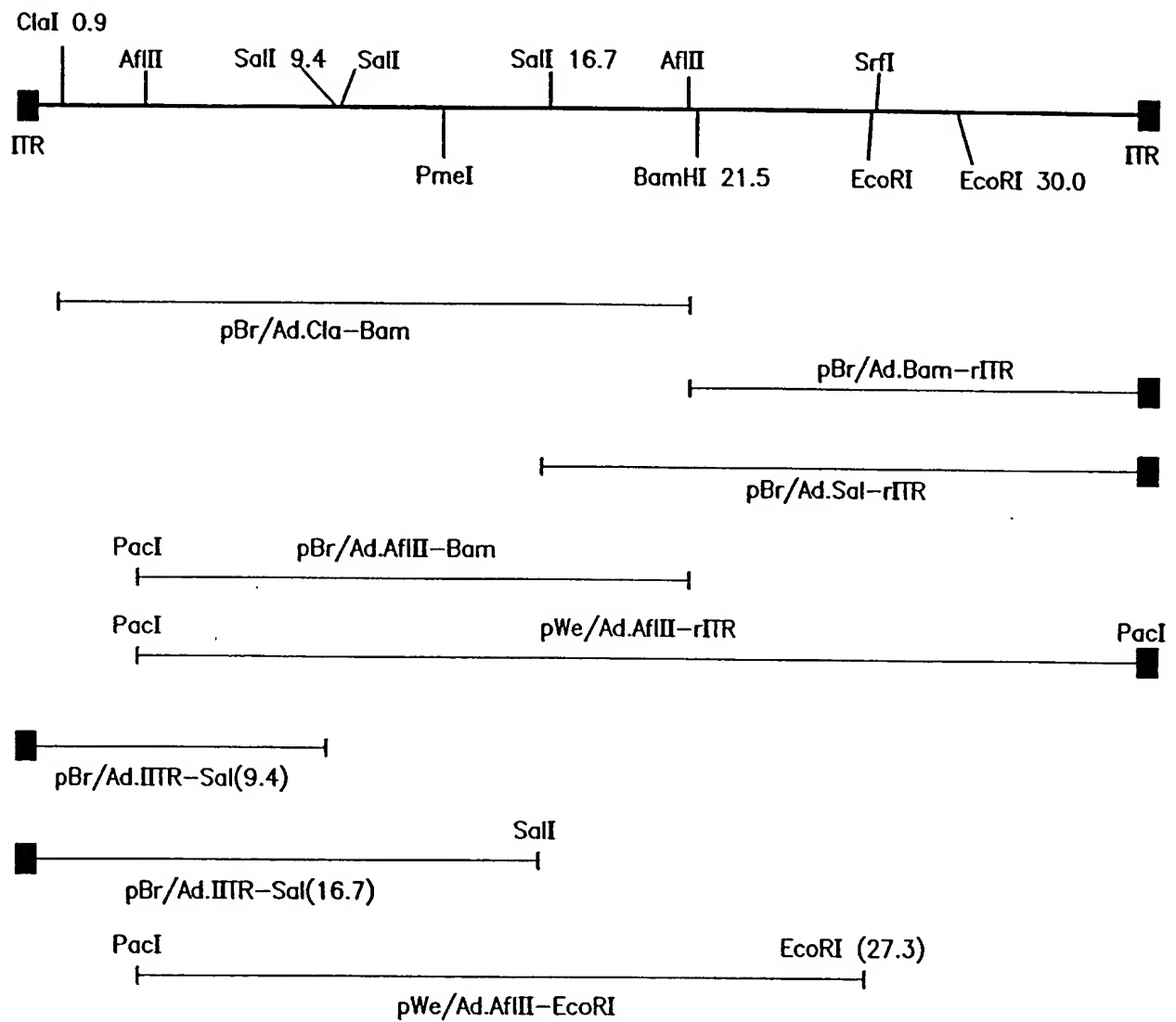


FIG. 20

# Adapter plasmid pAd5/L420-HSA

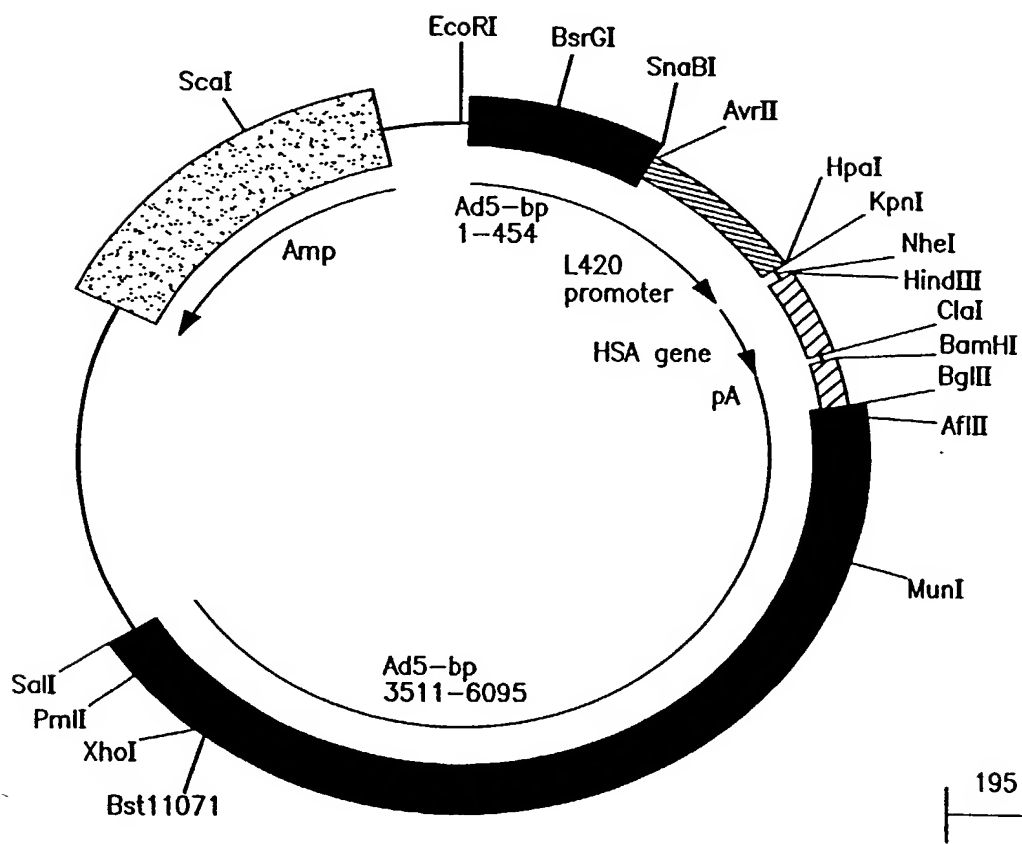


FIG. 21

[illegible]

FIG. 22



# Generation of recombinant adenoviruses

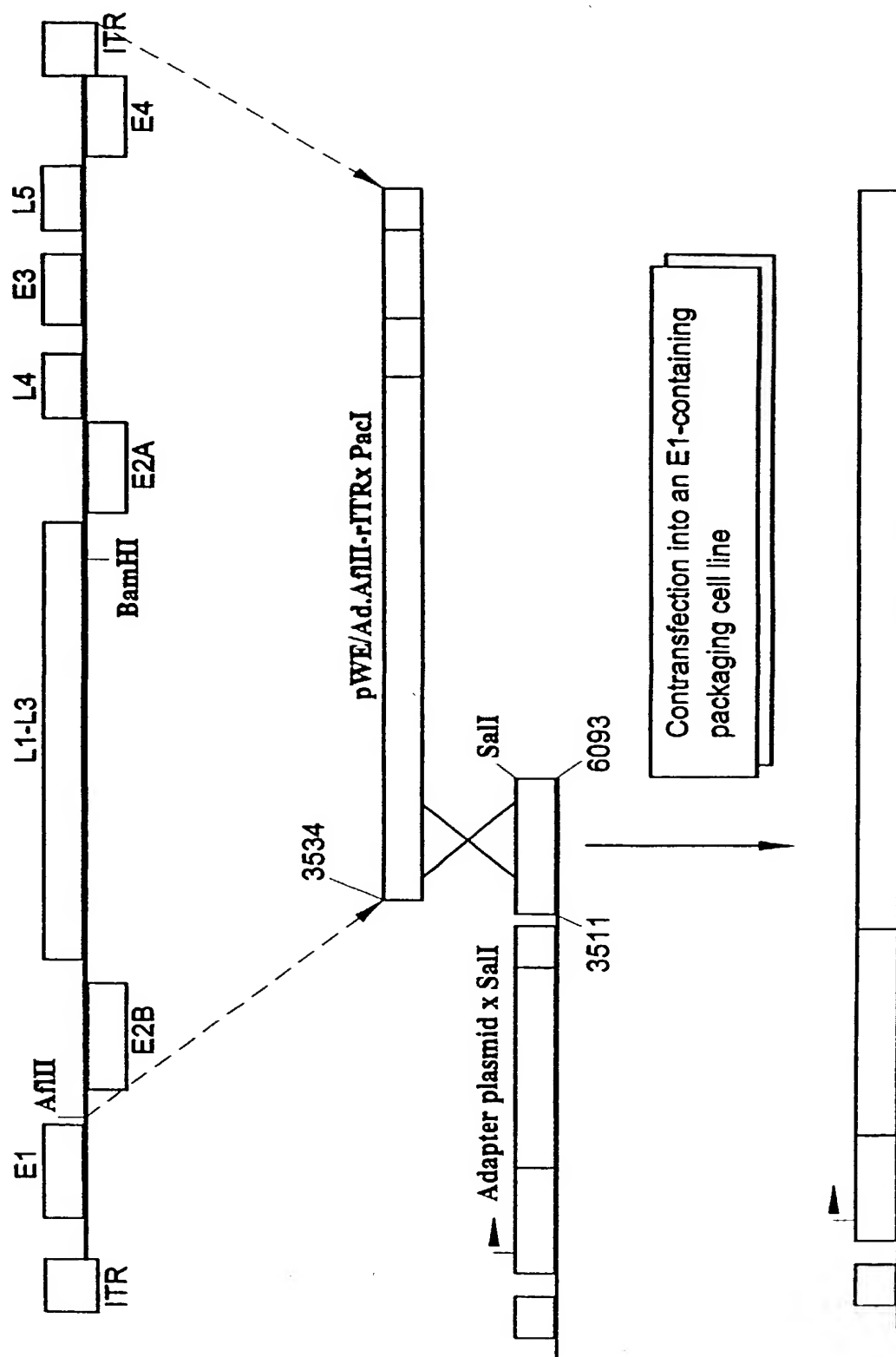
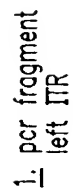


FIG. 23





**FIG. 25**

FIG. 26A

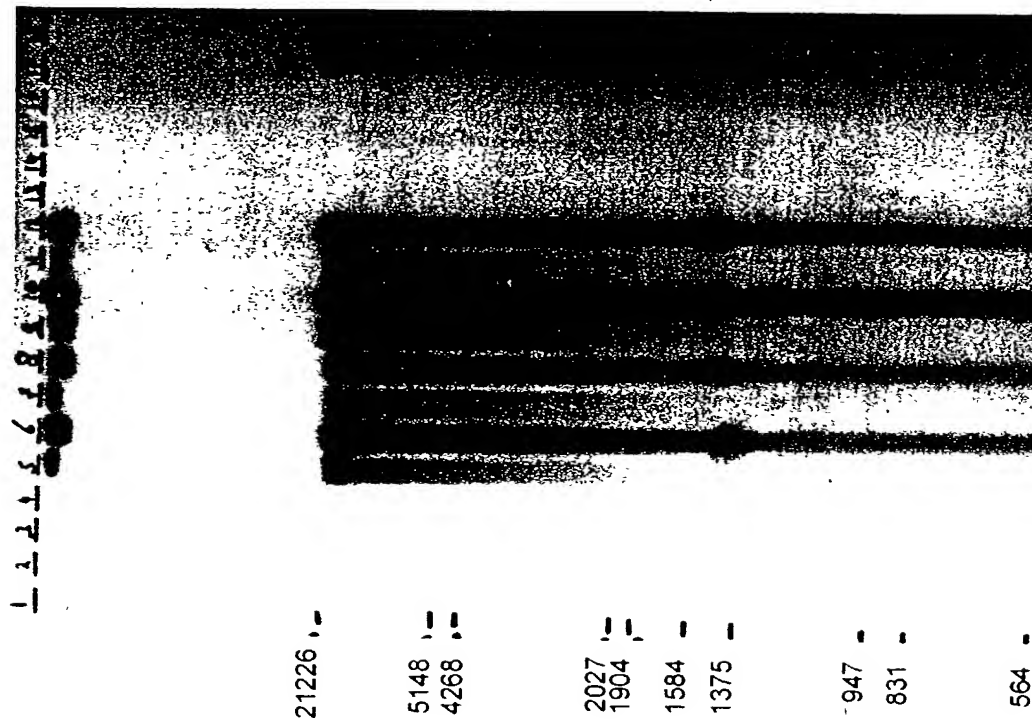


FIG. 26B

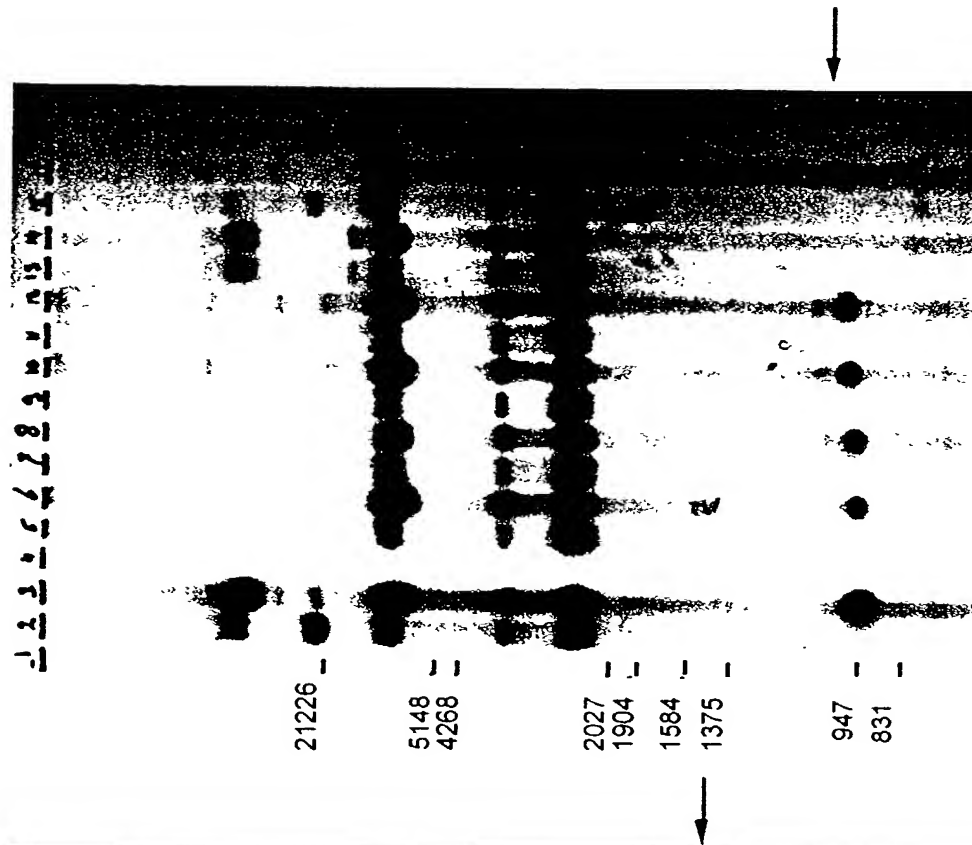
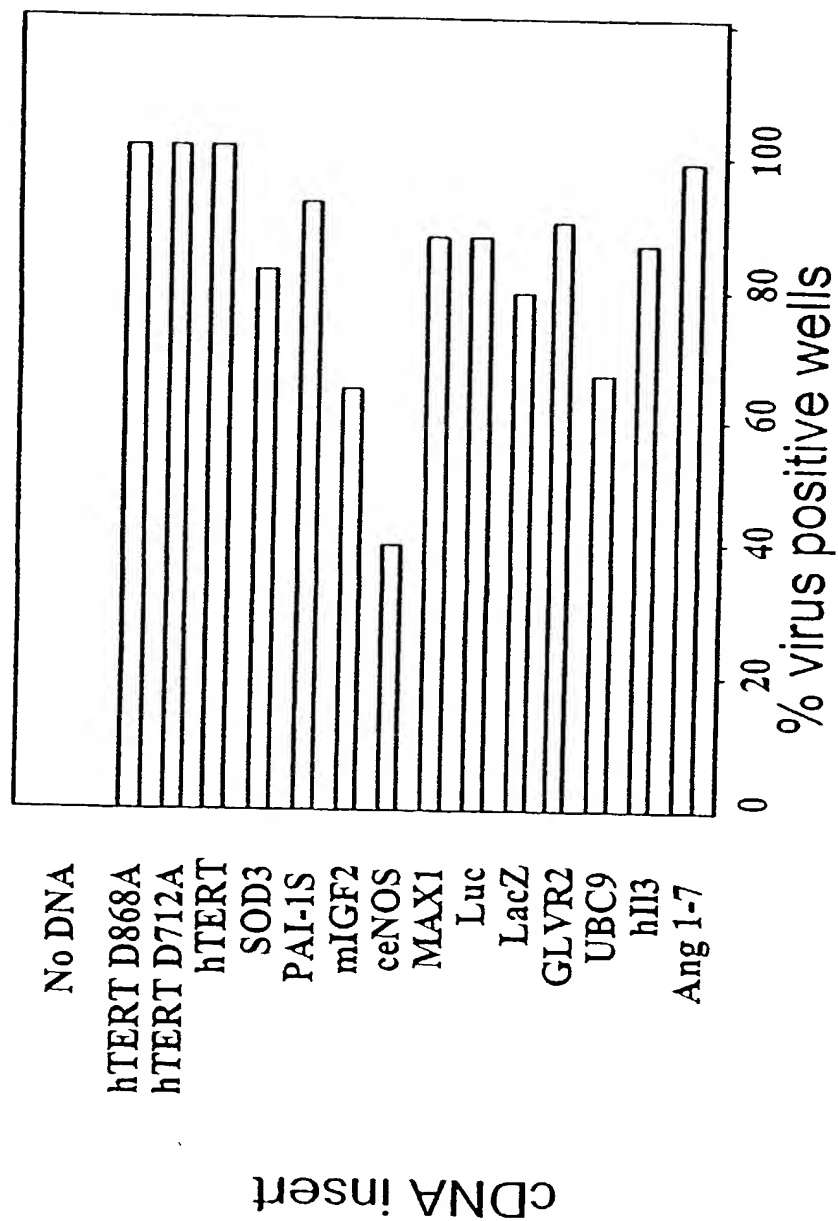


FIG. 26C

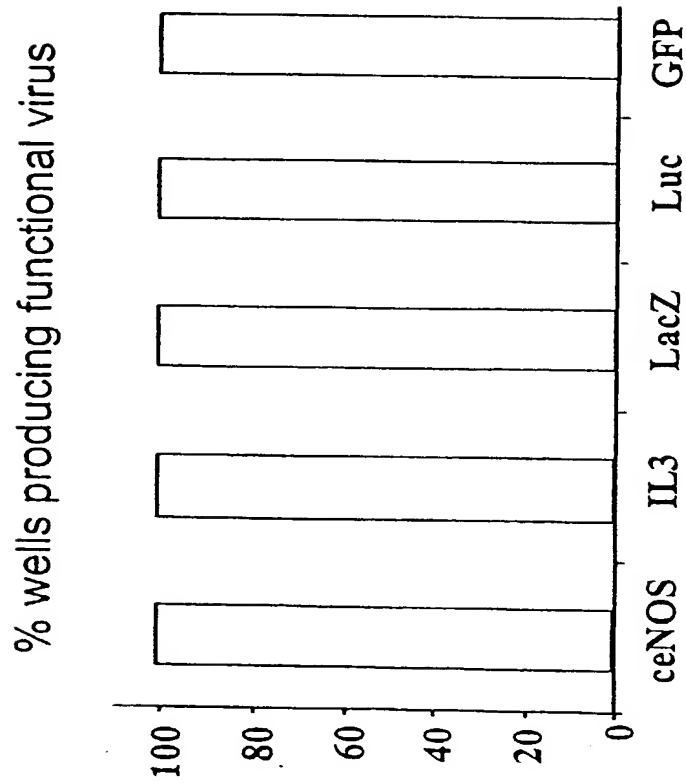


Average percentage CPE efficiency: 86 %

FIG. 27

Gene	Insert kb	Average titer 0.8 ±0.7 x 10 <sup>9</sup> pfu/ml
• ceNOS	3.6	
• hTERT	3.5	
• hTERT D712A	3.5	
• lacZ	3.2	
• hCAT1	2.2	
• GLVR2	2.0	
• Luc	1.7	
• SOD3	1.4	
• MAX1	.550	
• hVEGF121	.511	
• hIL3	.434	
• UBC9	.412	
• ANG1-7	.104	

FIG. 28



Gene	Number of CPE+ wells
ceNOS	19/19
IL3	7/7
lacZ	36/36
Luc	40/40
GFP	48/48

Gene	Number of plaques
ceNOS	9/9
IL3	9/9
lacZ	40/40
Luc	9/9
EGFP	IP
GLVR2	9/9

FIG. 29



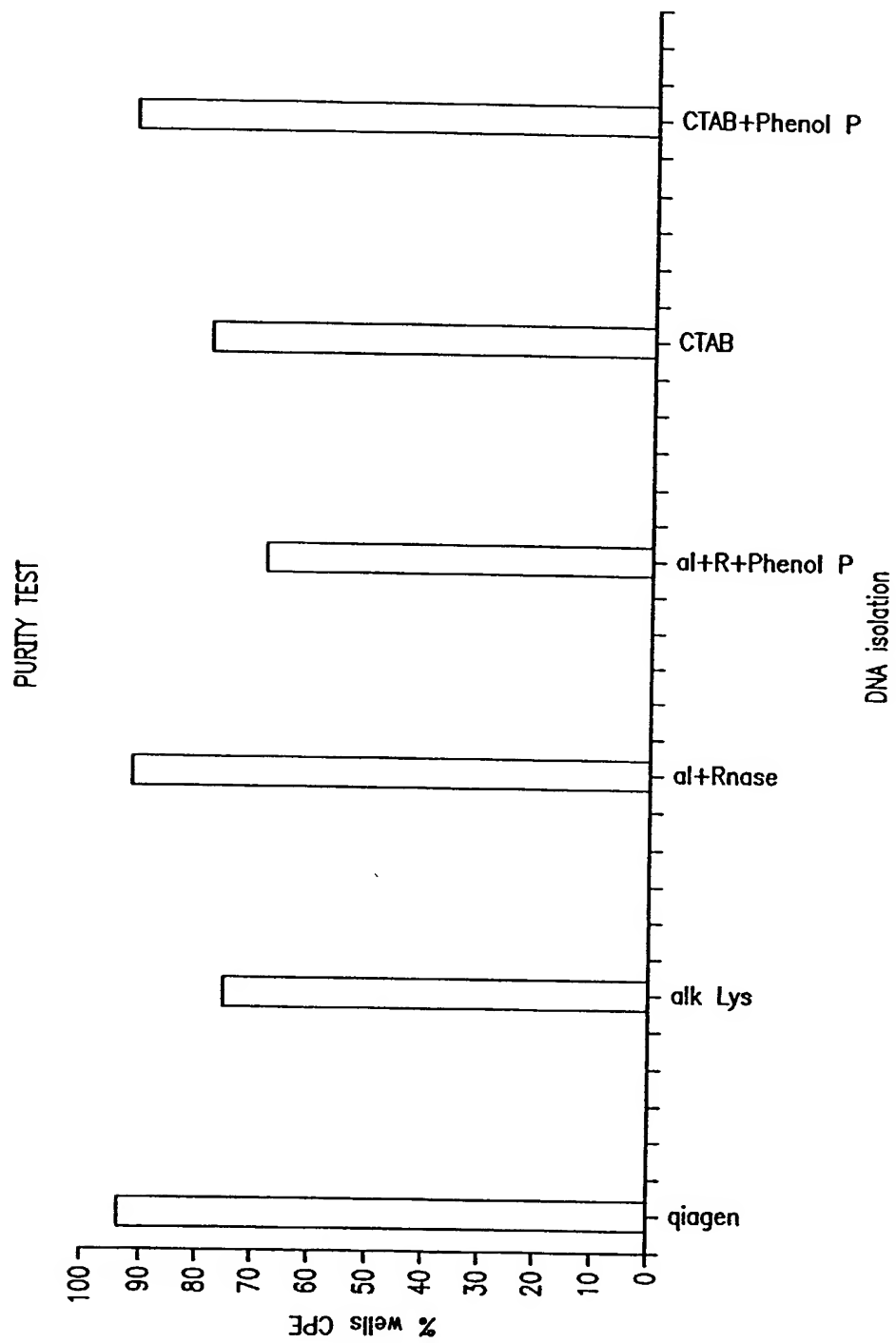


FIG. 30

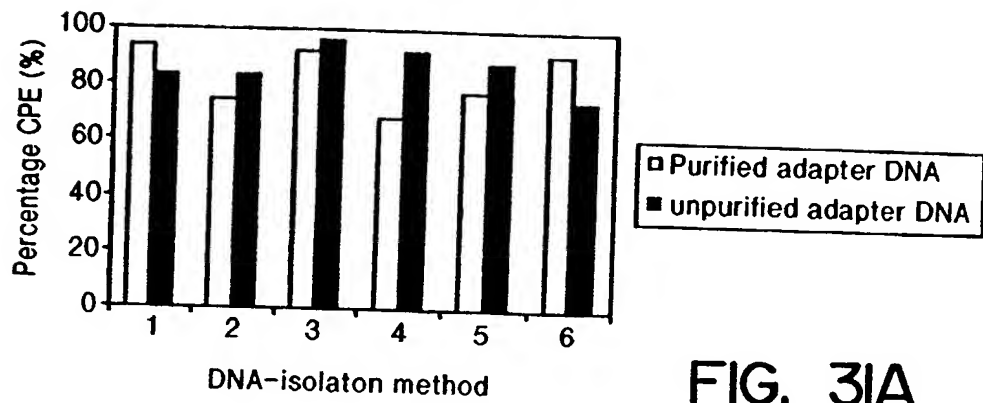


FIG. 3IA

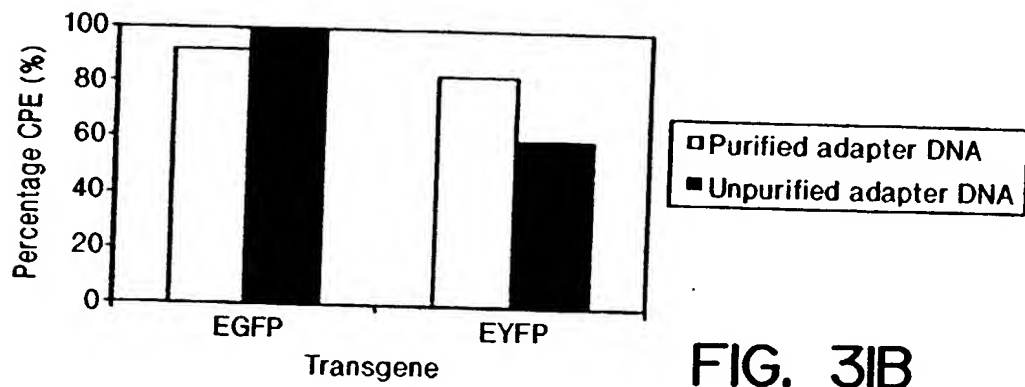


FIG. 3IB

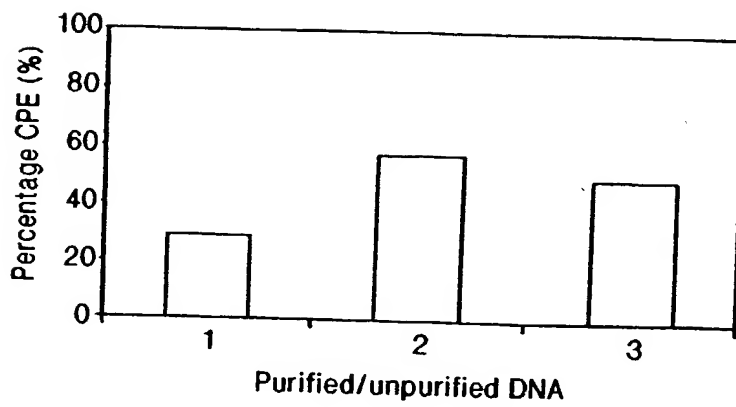
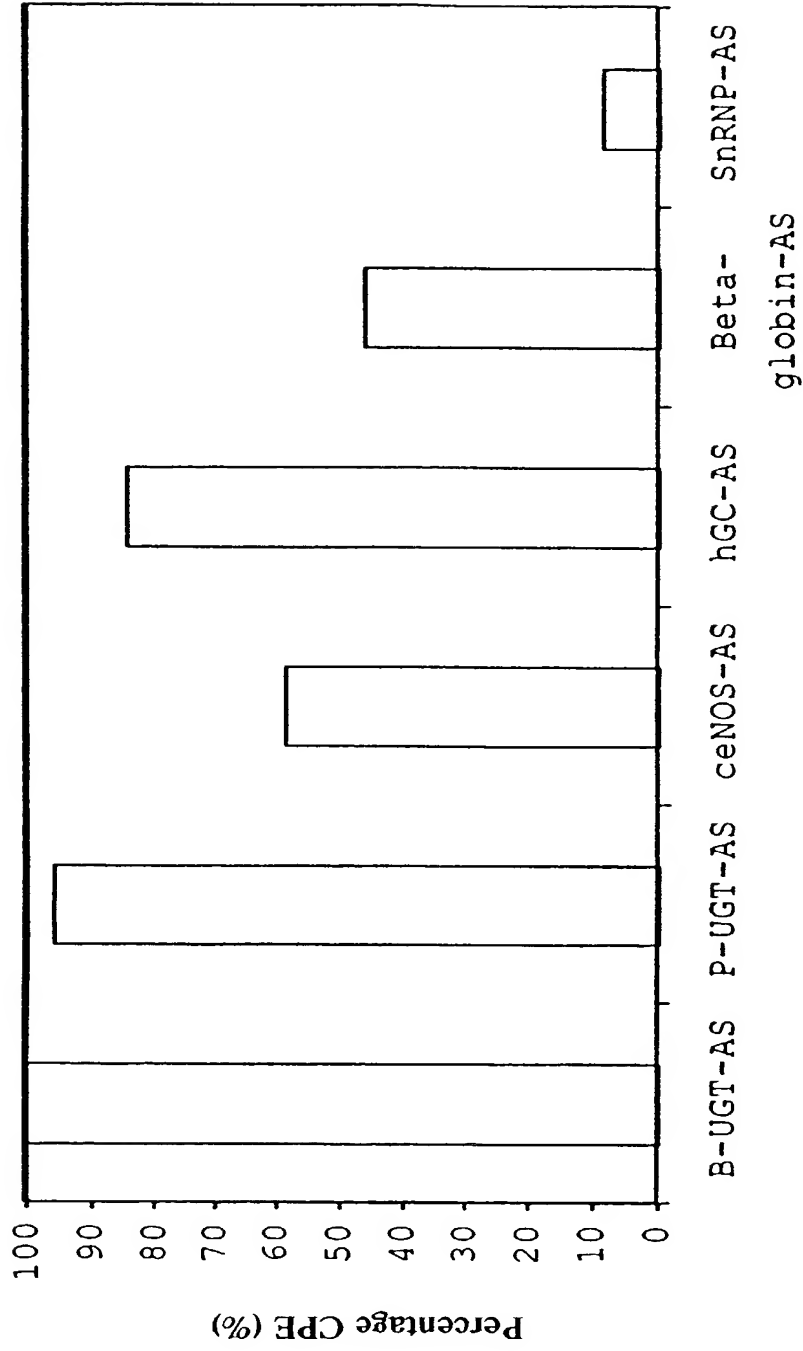


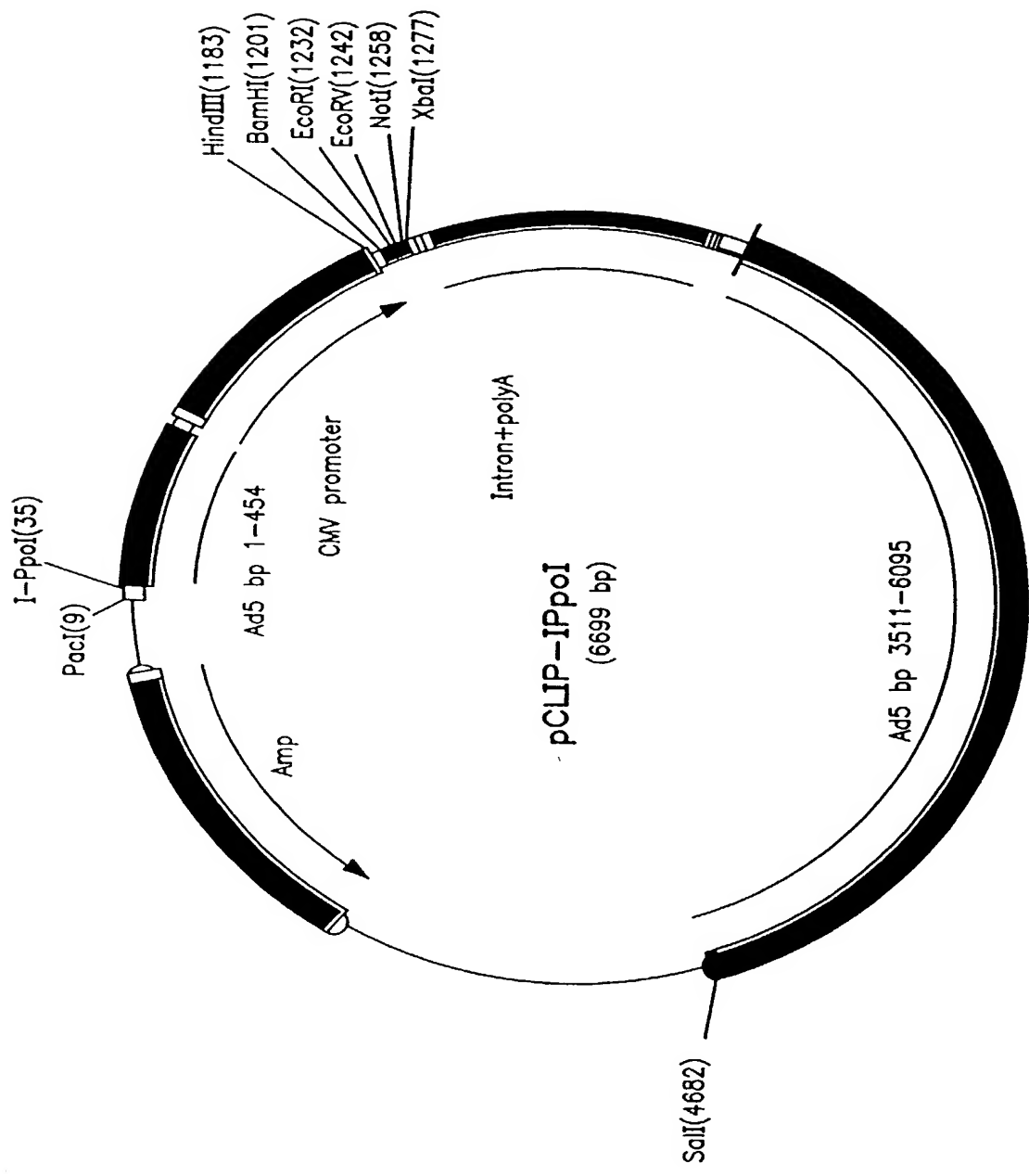
FIG. 3IC





AS-virus

FIG. 33



**FIG. 34A**

FIG. 34A

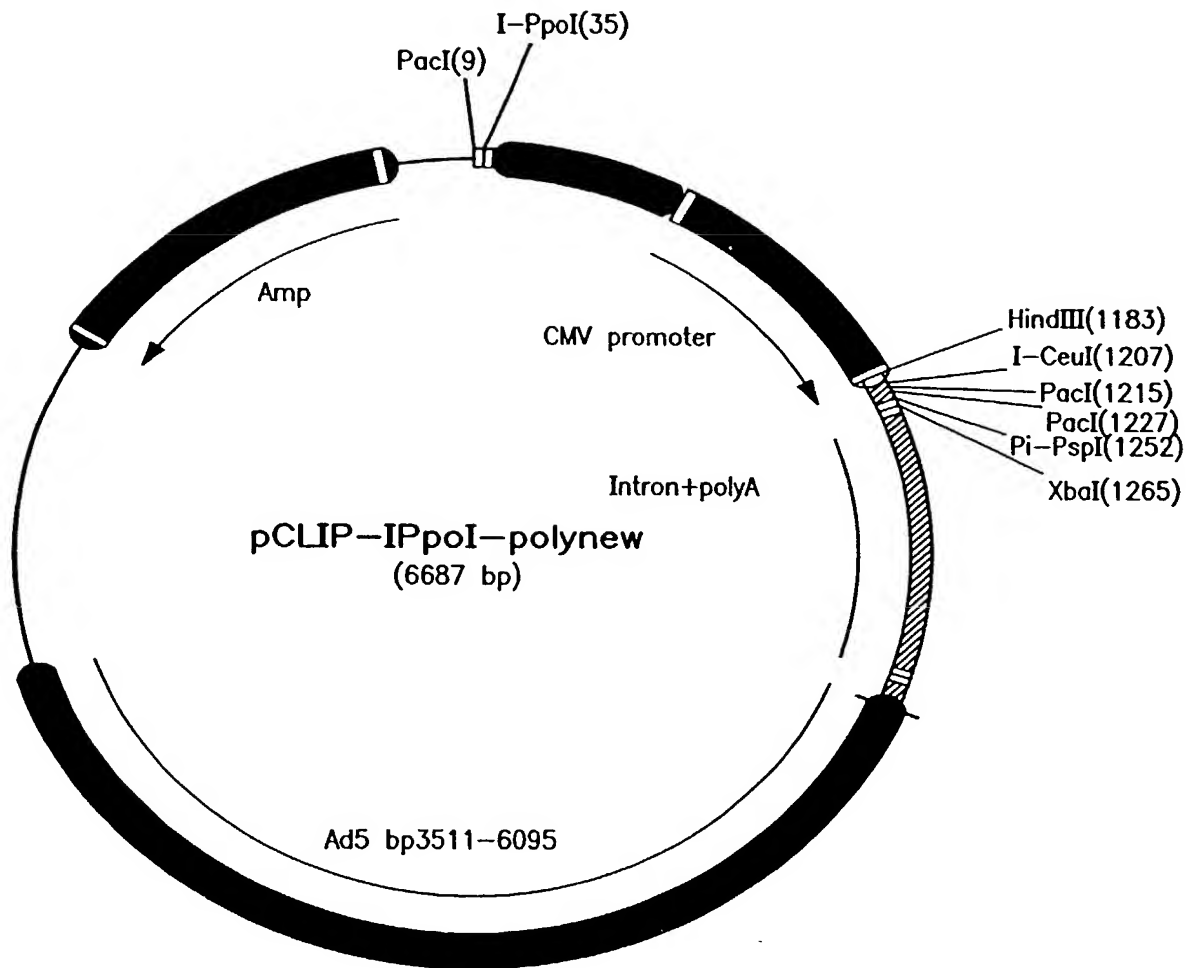


FIG. 34B

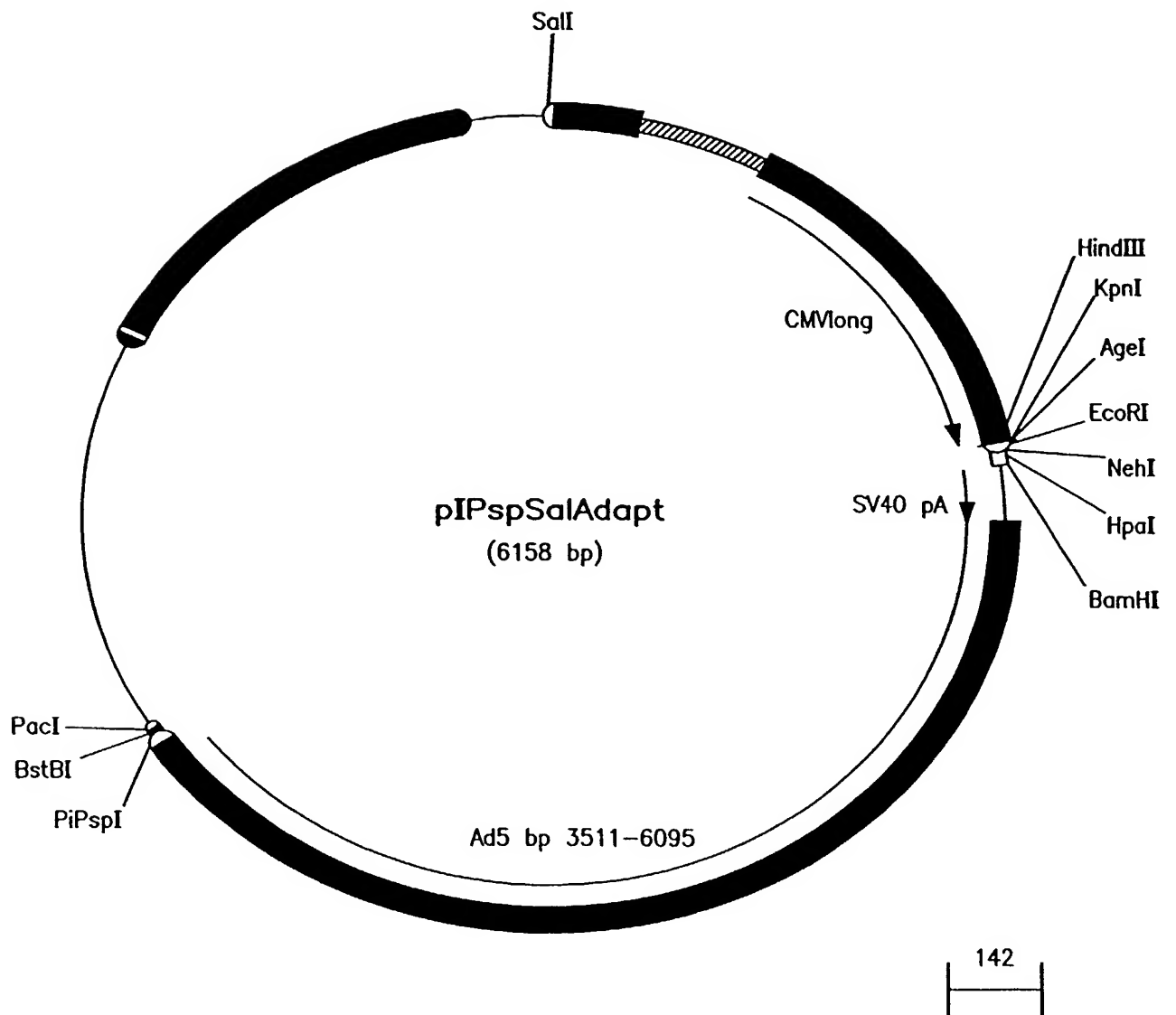


FIG. 34C

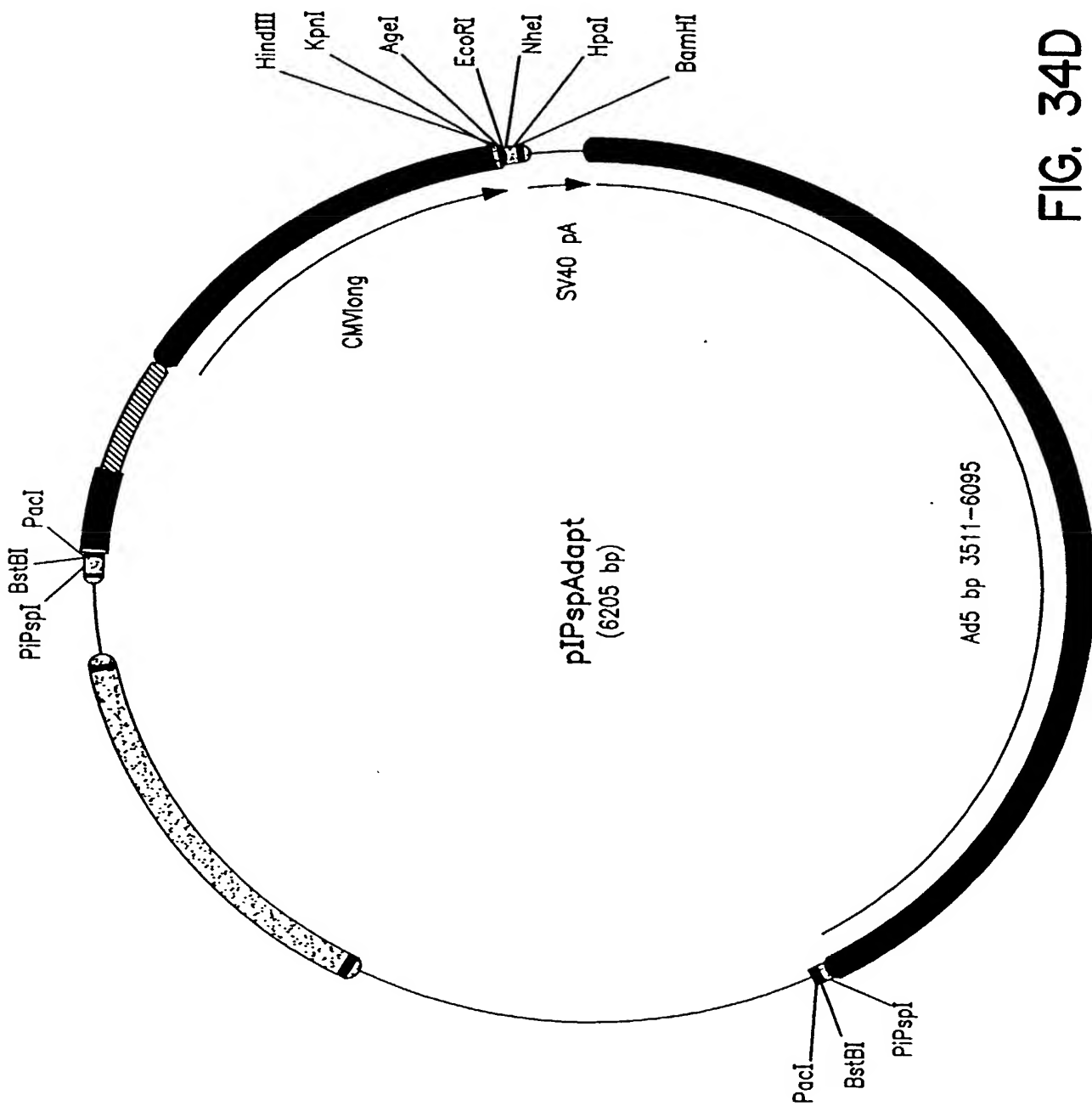


FIG. 34D



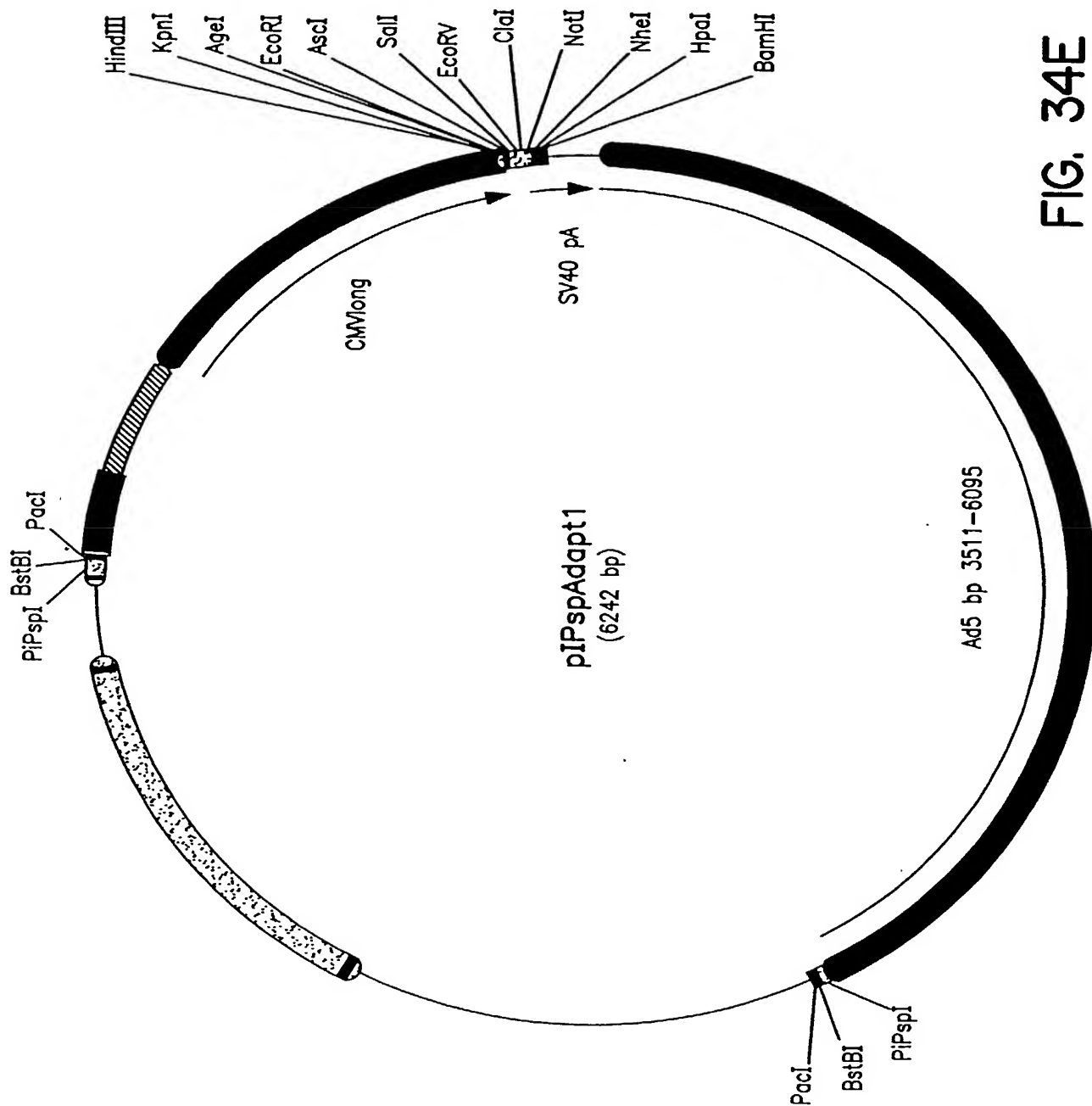


FIG. 34E

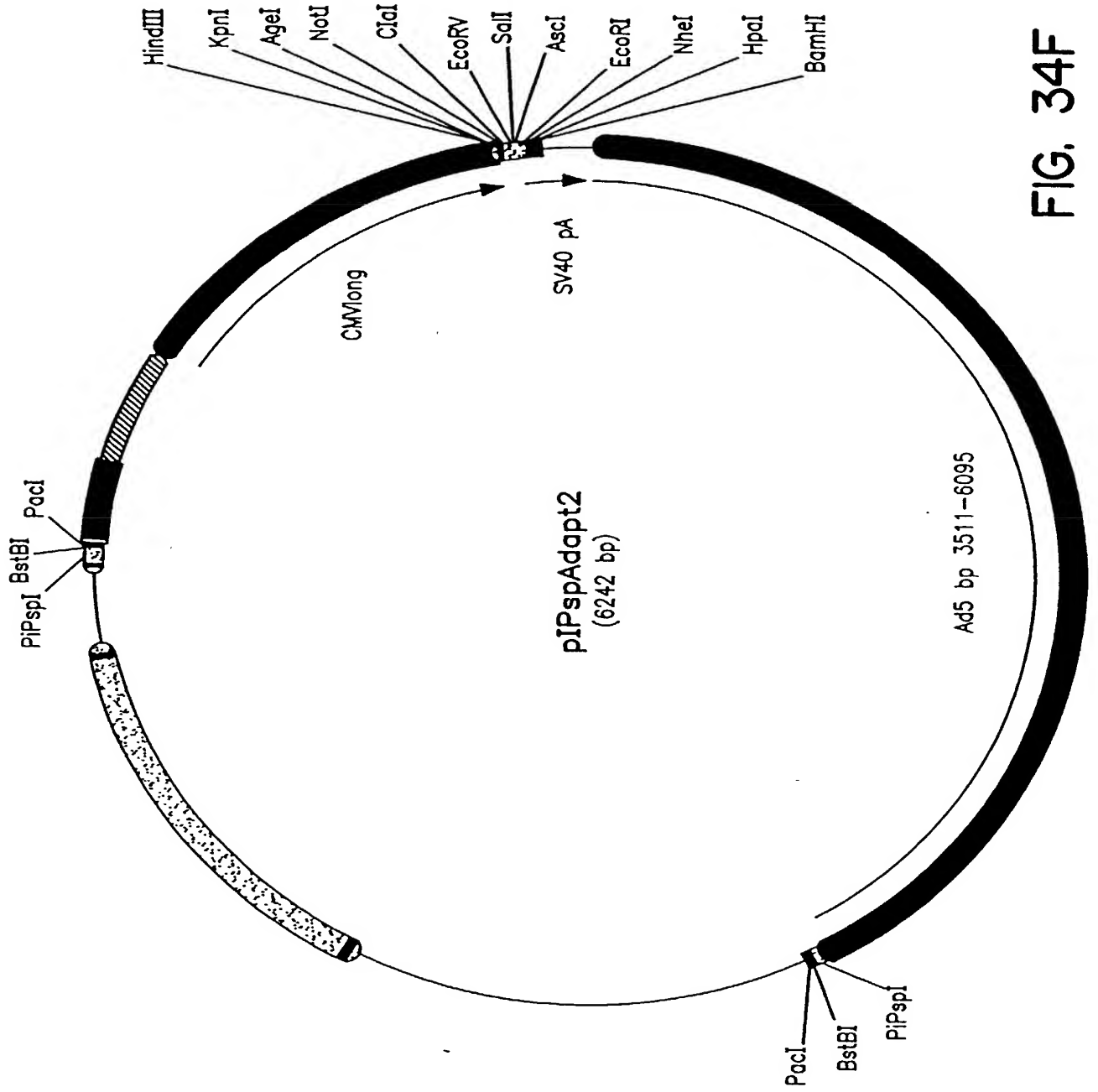


FIG. 34F

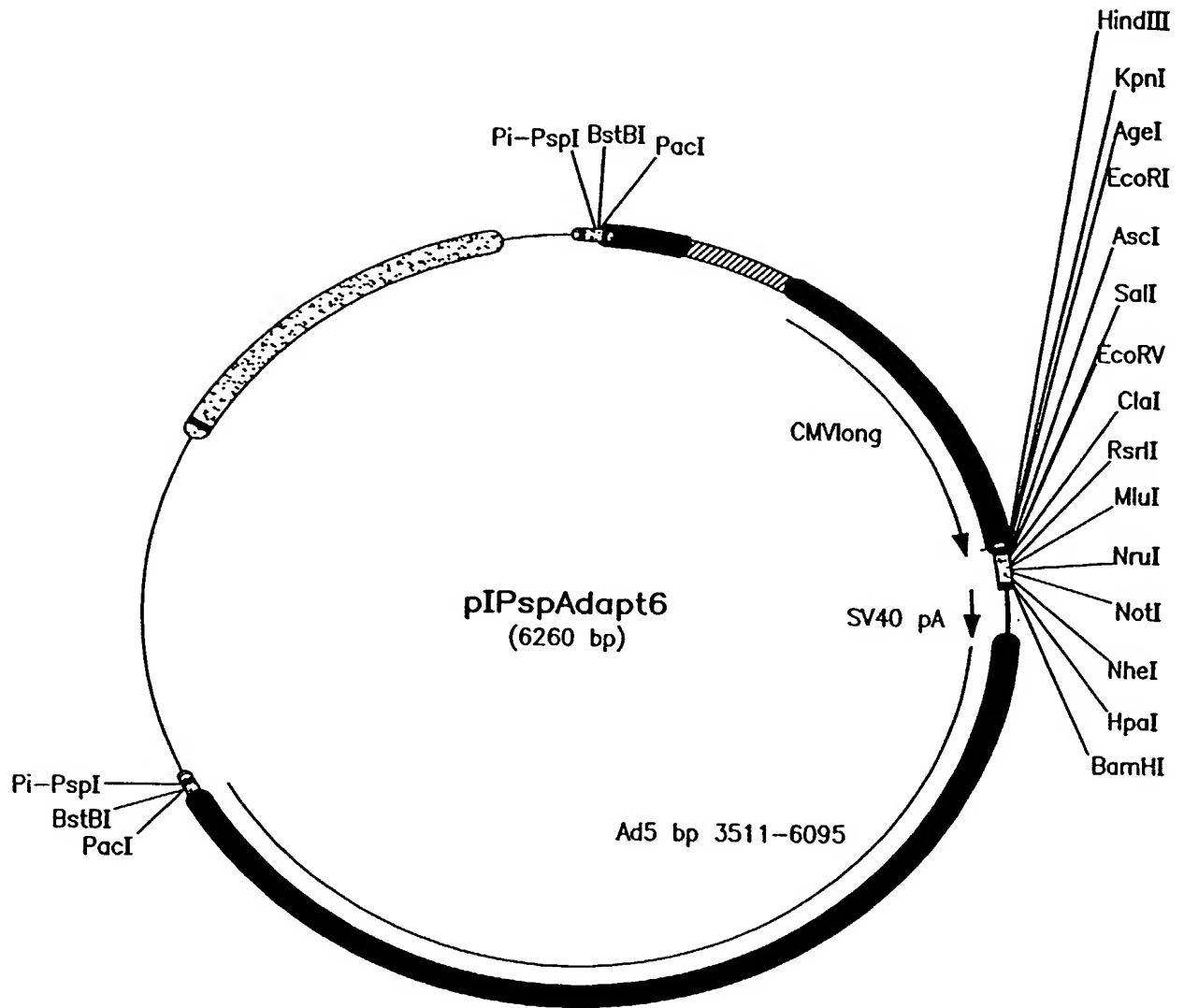


FIG. 34G

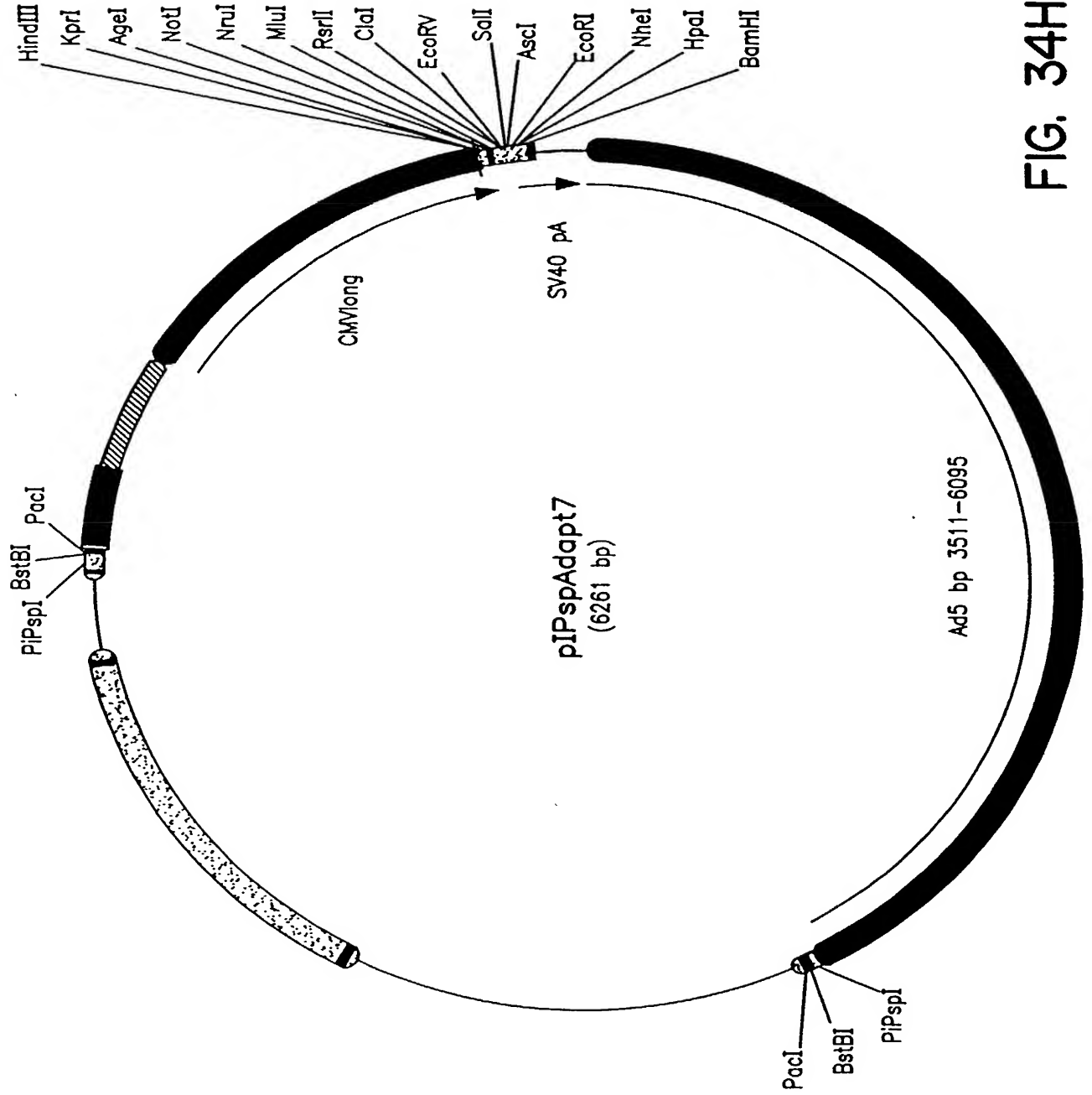


FIG. 34H

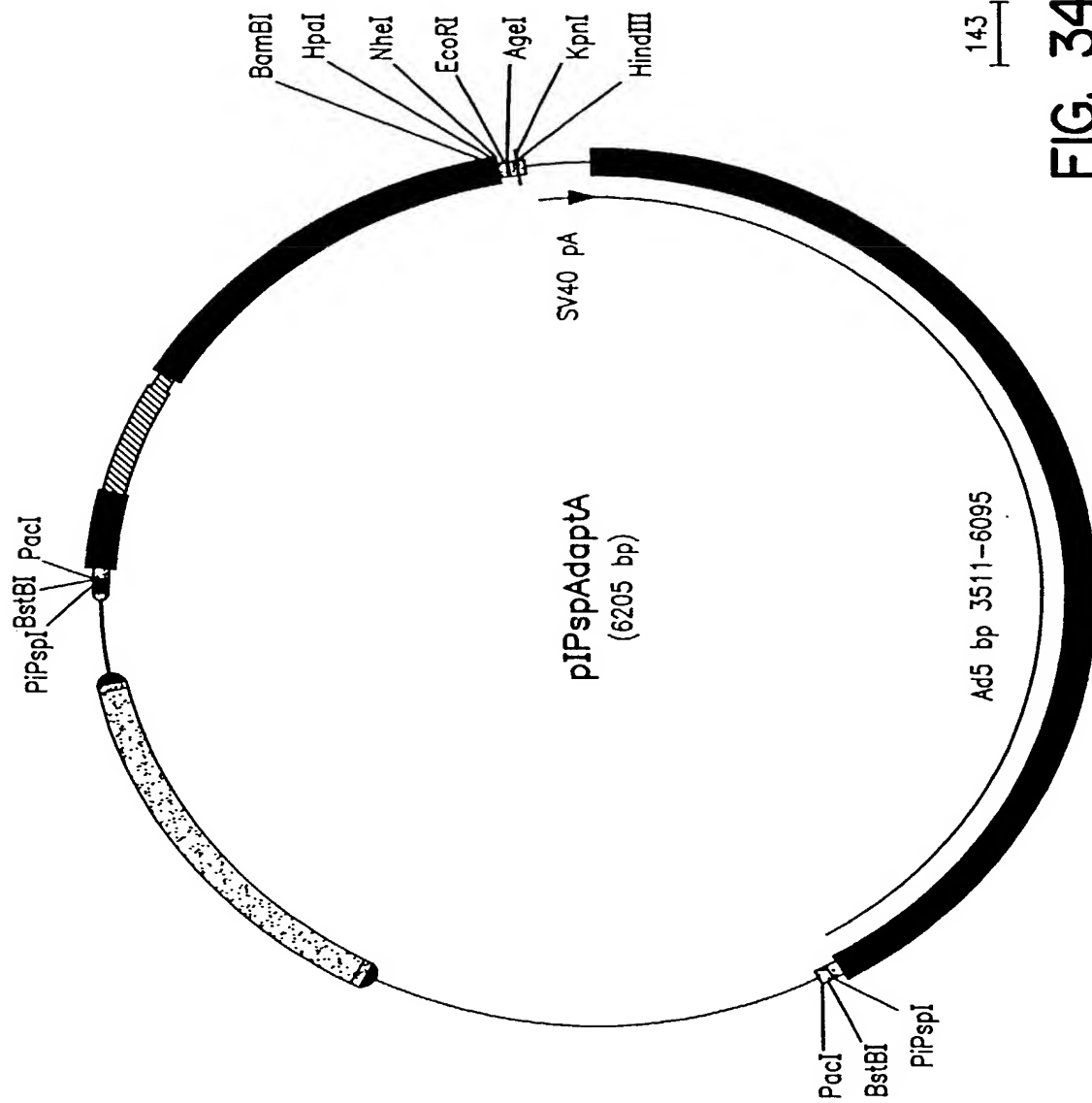


FIG. 34 I

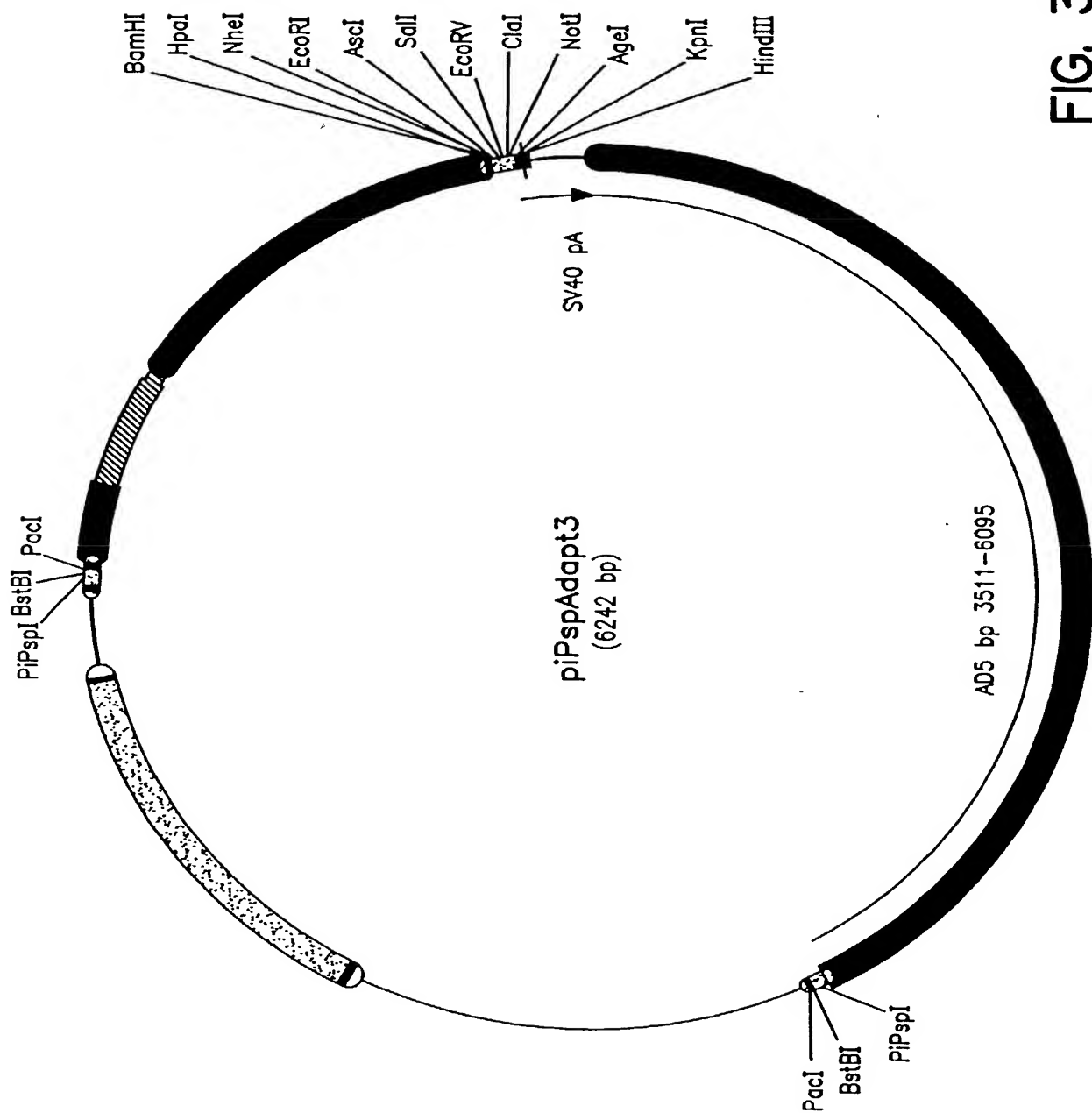


FIG. 34J

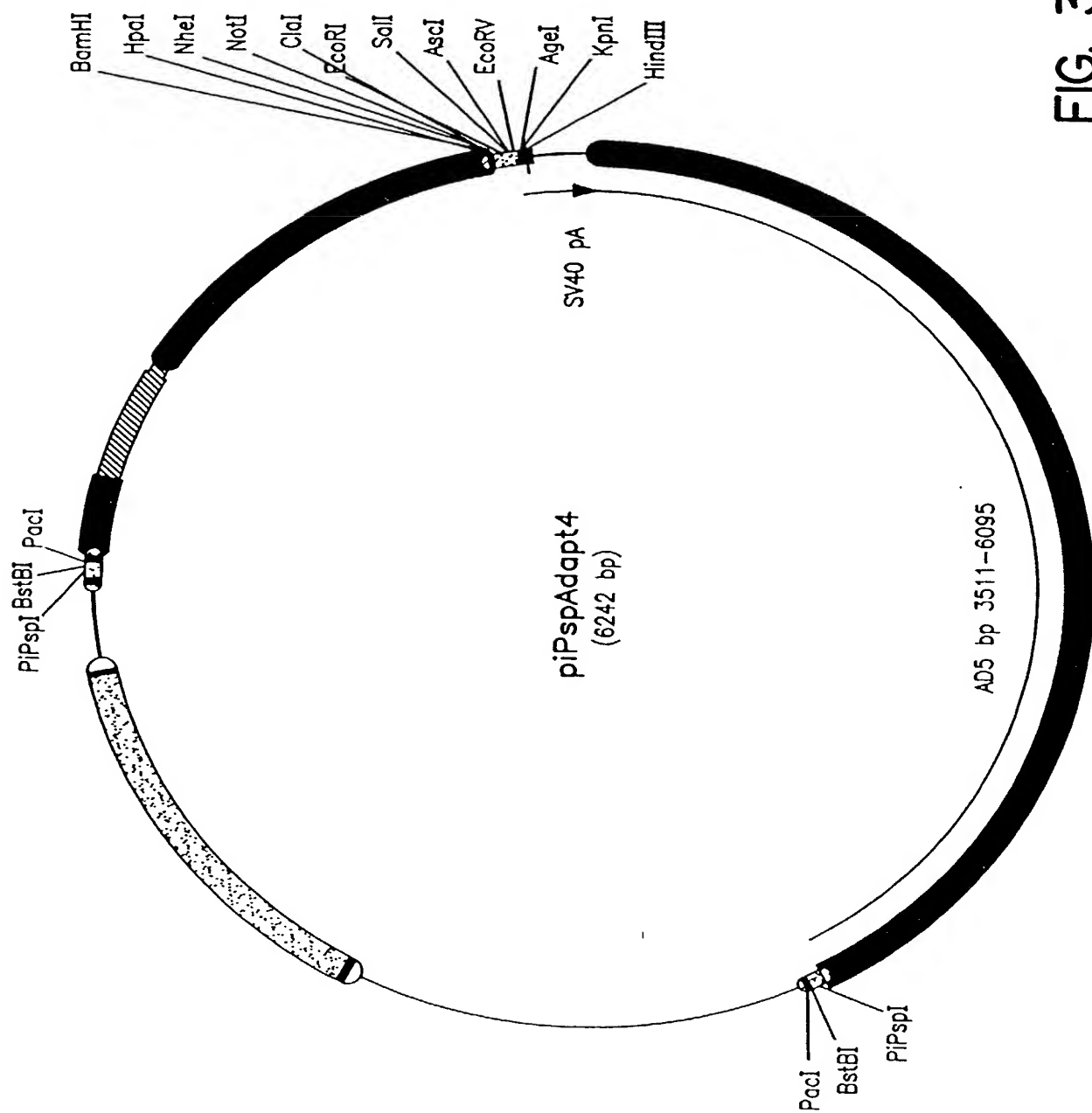
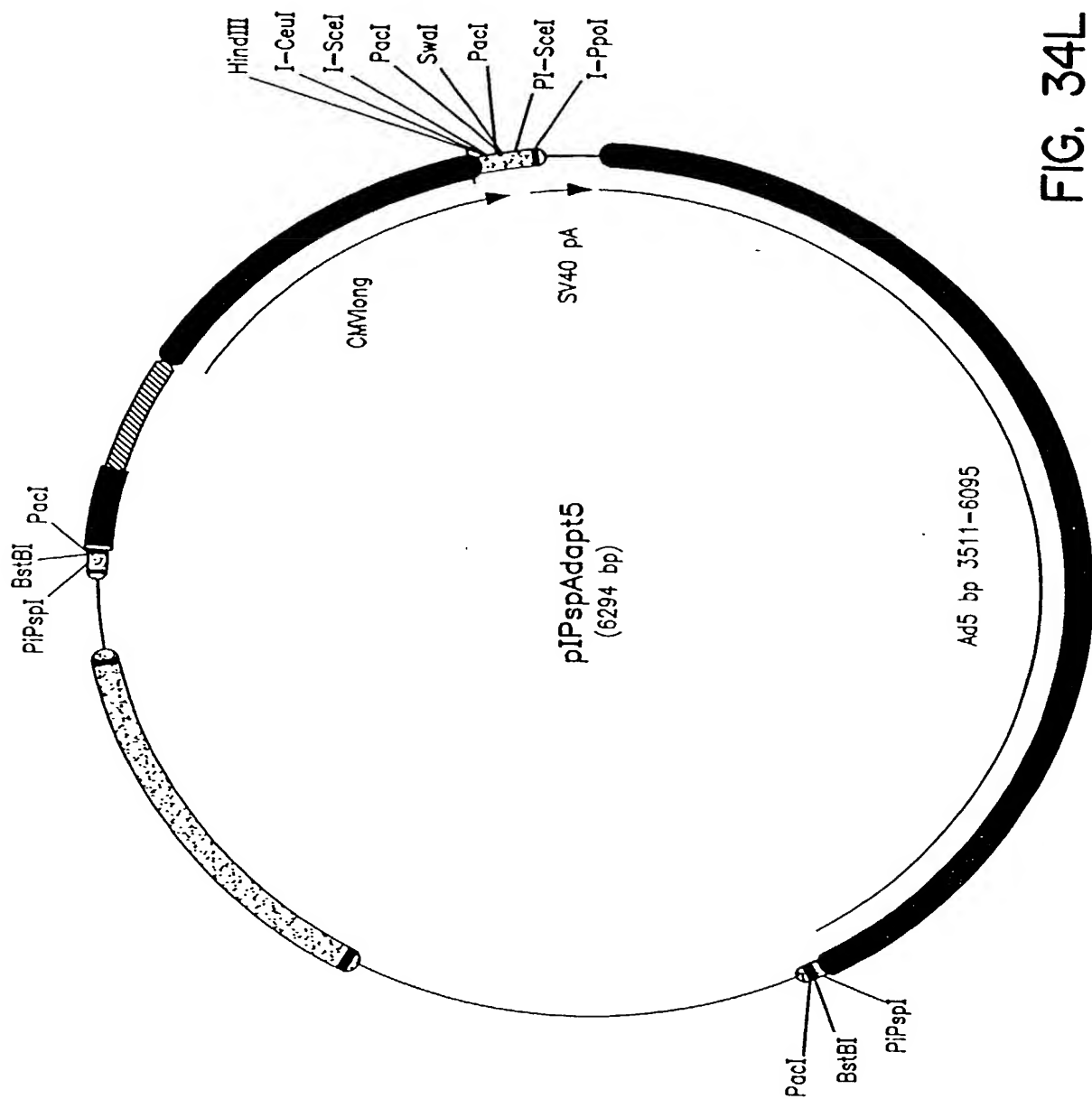


FIG. 34K



**FIG. 34L**



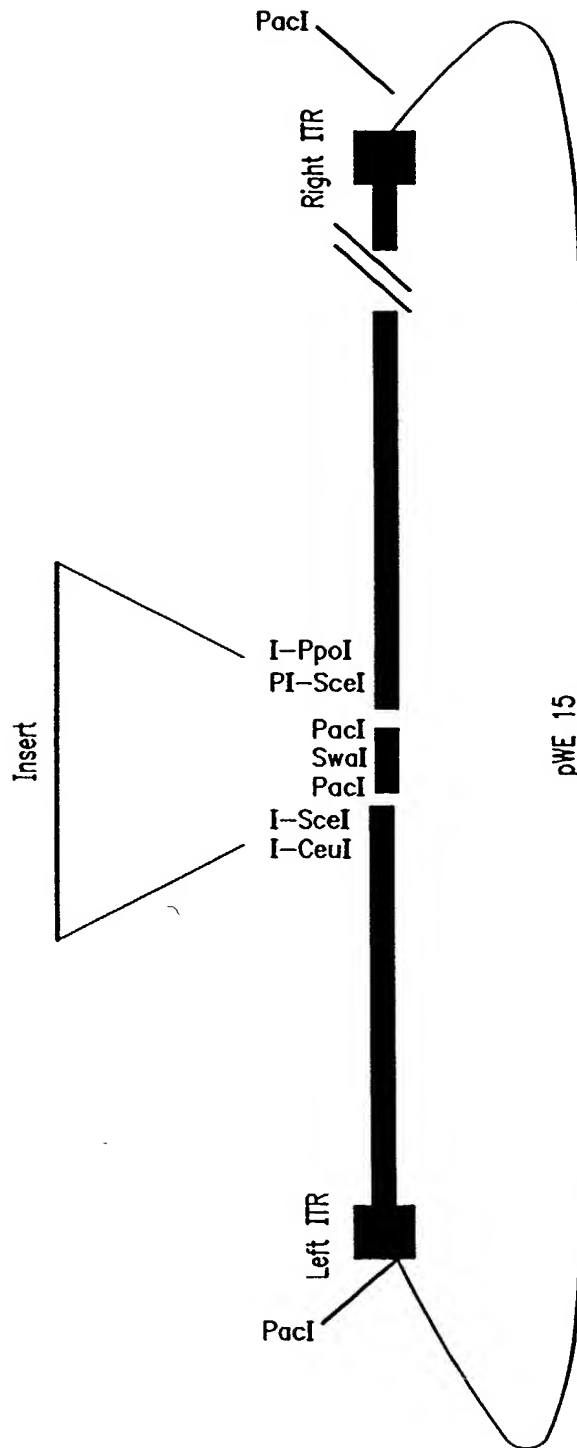


FIG. 34M

Relative amounts of wells with CPE after transfection of PER.C6/E2A cells with pCLIP-LacZ and the adapter plasmid pIPspAdapt2.

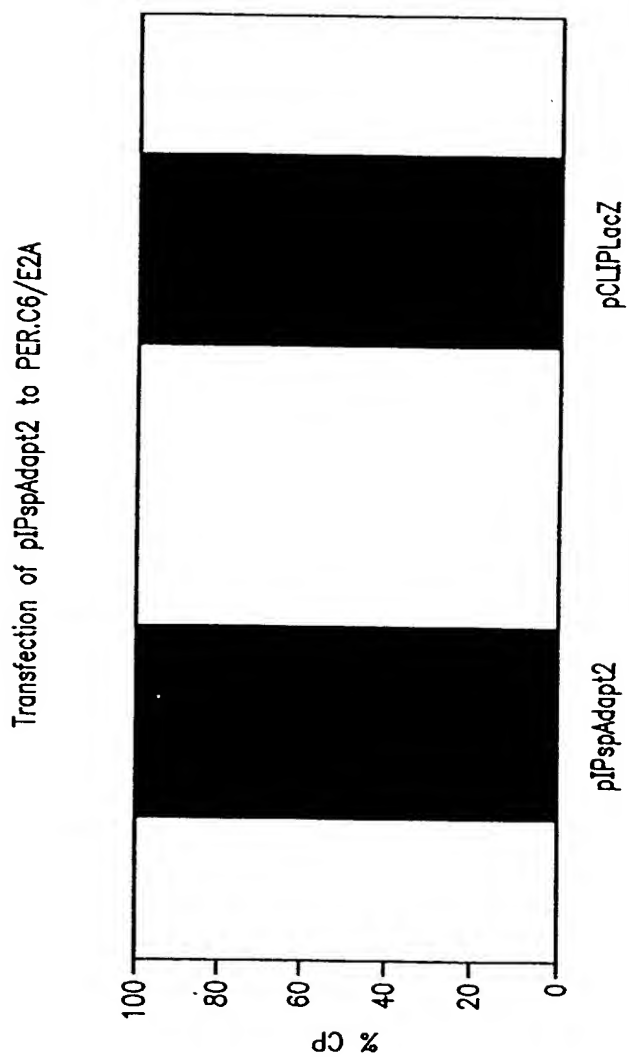


FIG. 34N

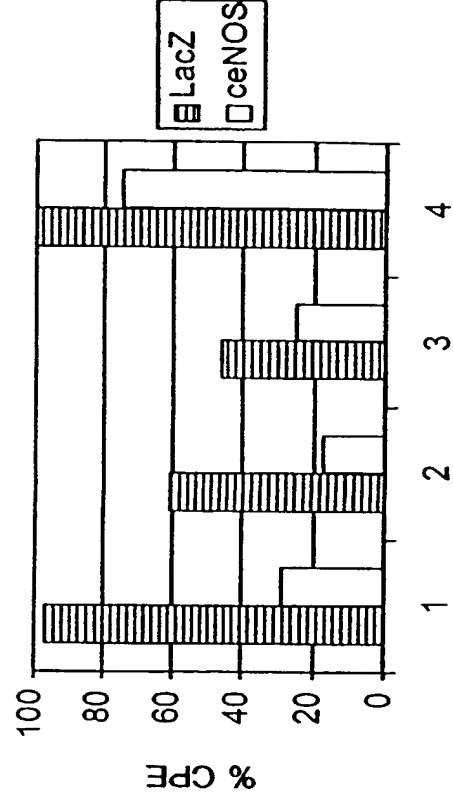


FIG. 35

FIG. 35 is a bar chart showing the percentage of CPE for LacZ and ceNOS across four categories (1, 2, 3, 4). The y-axis represents % CPE from 0 to 100. The x-axis represents the categories. For each category, there are two bars: a hatched bar for LacZ and a white bar for ceNOS. The data shows that LacZ generally has a higher % CPE than ceNOS in categories 1, 2, and 4, while in category 3, ceNOS has a higher % CPE than LacZ.

# Construction total Adeno cDNA Library (1)

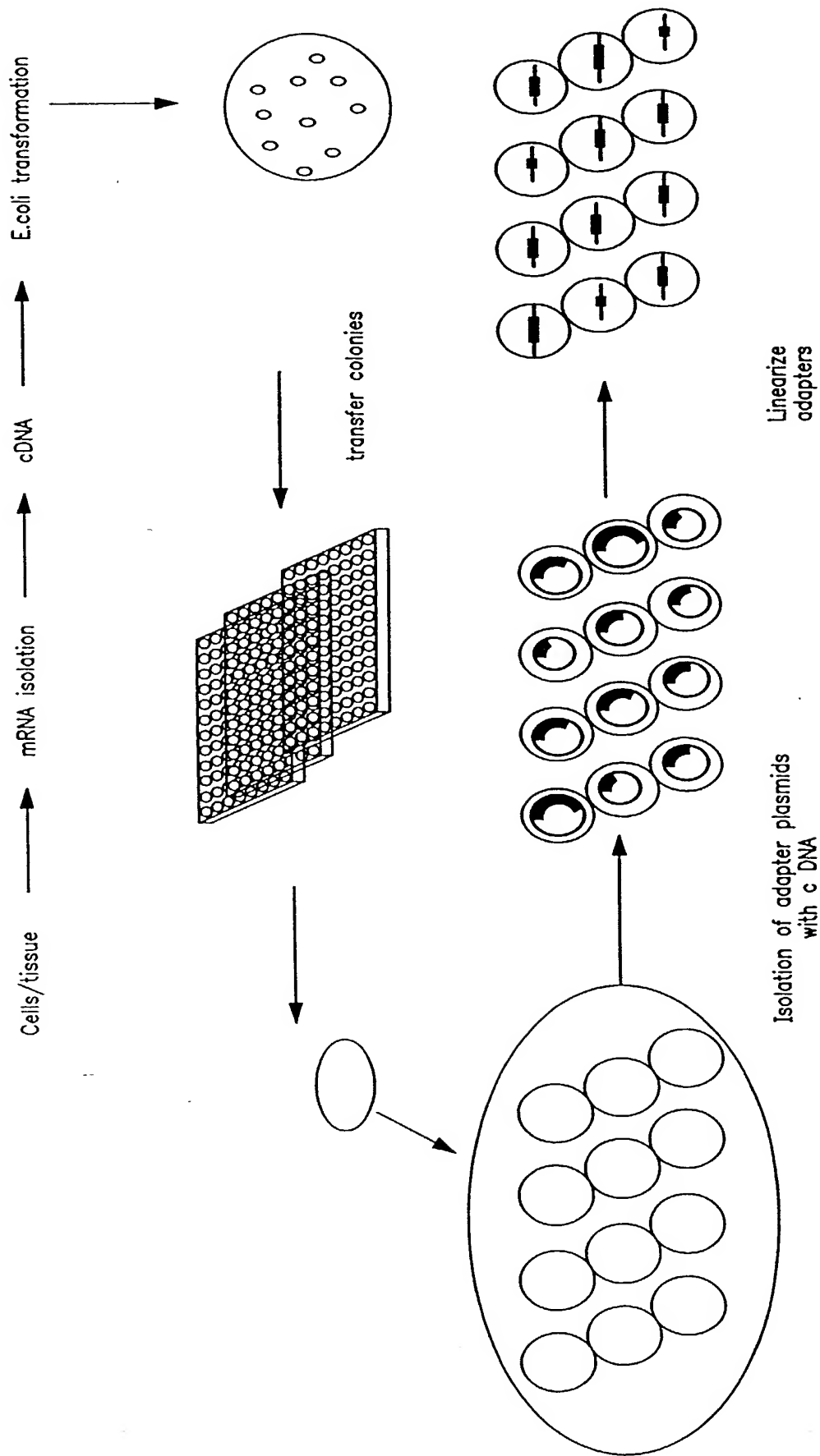


FIG. 36A

# Construction total Adeno cDNA Library (II)

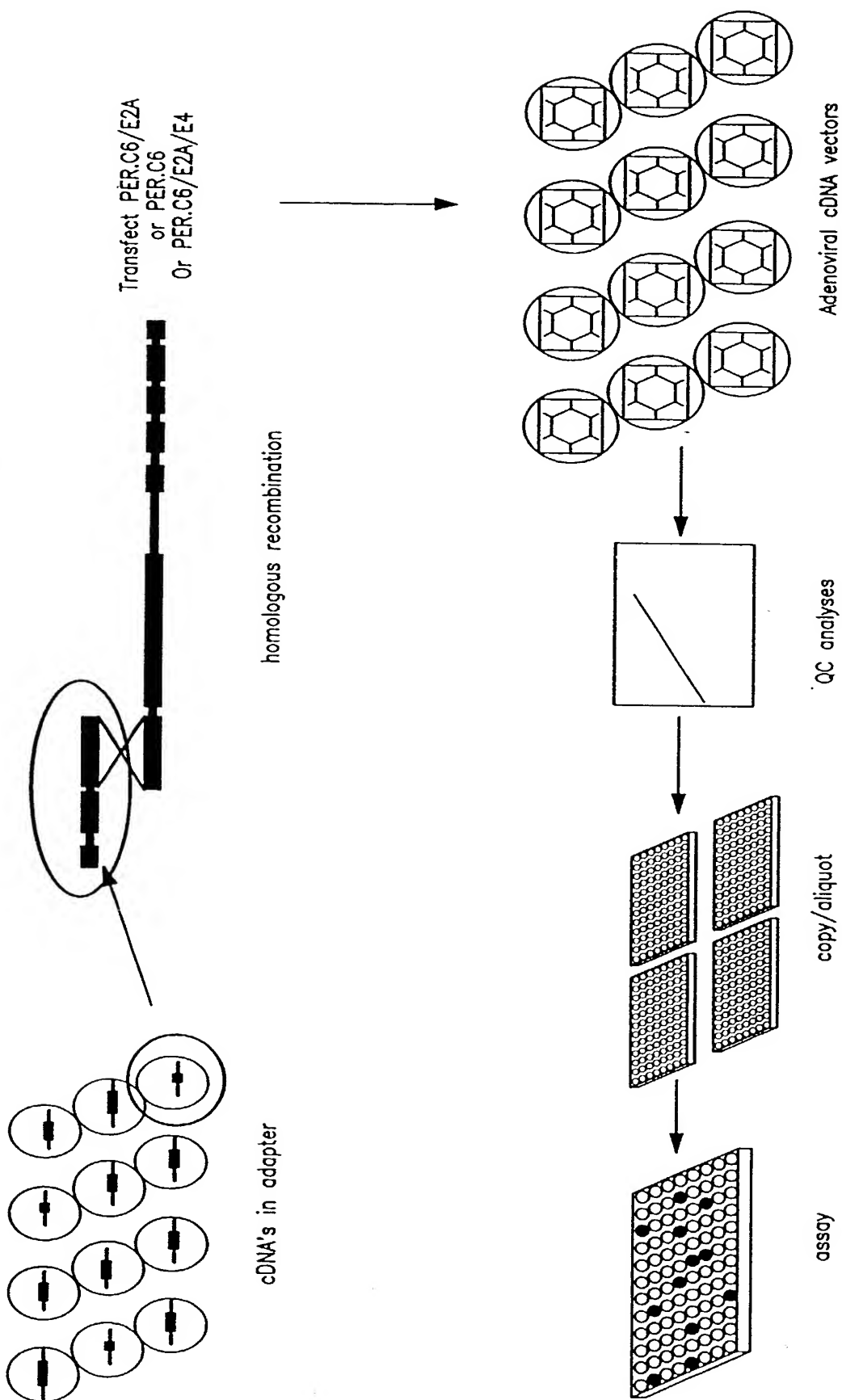


FIG. 36B

# EXAMPLE 21 384 WELL PLATE IN PROGRESS

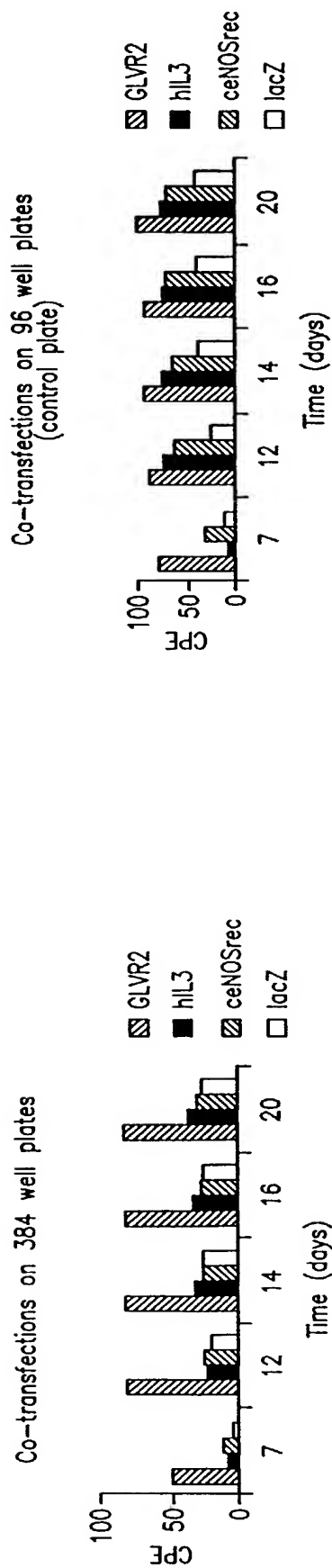


FIG. 37A

FIG. 37B

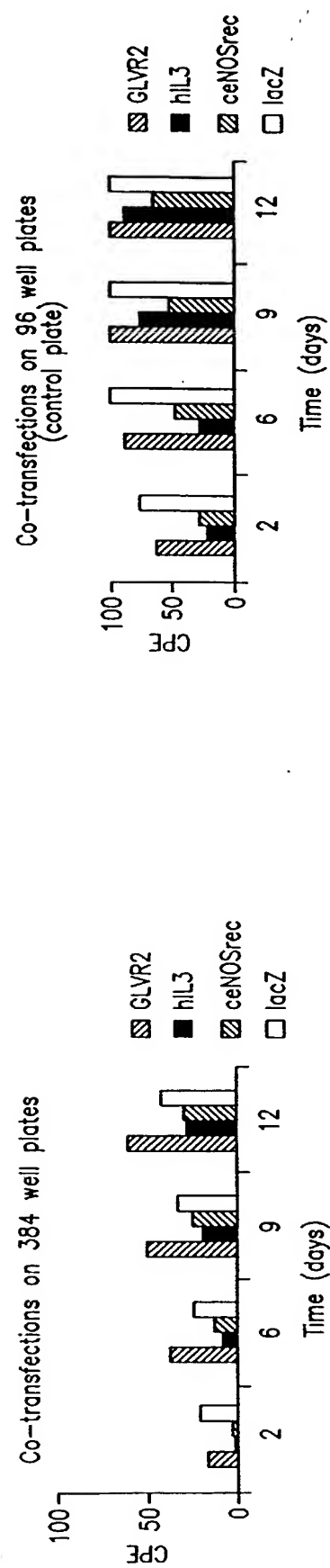


FIG. 37C

FIG. 37D

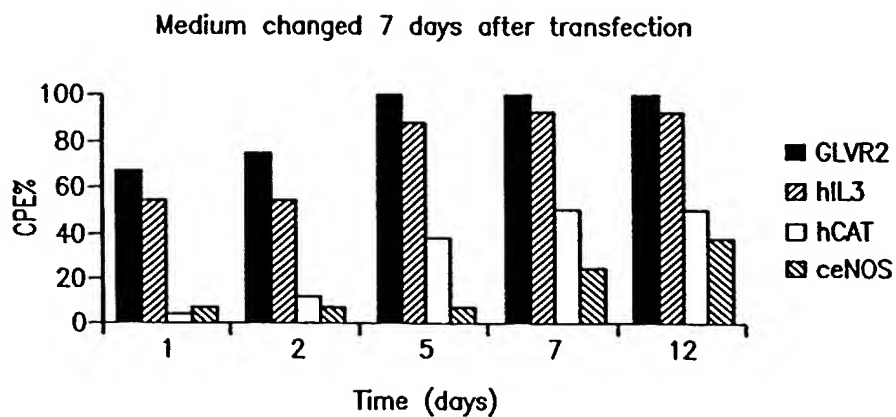


FIG. 38A

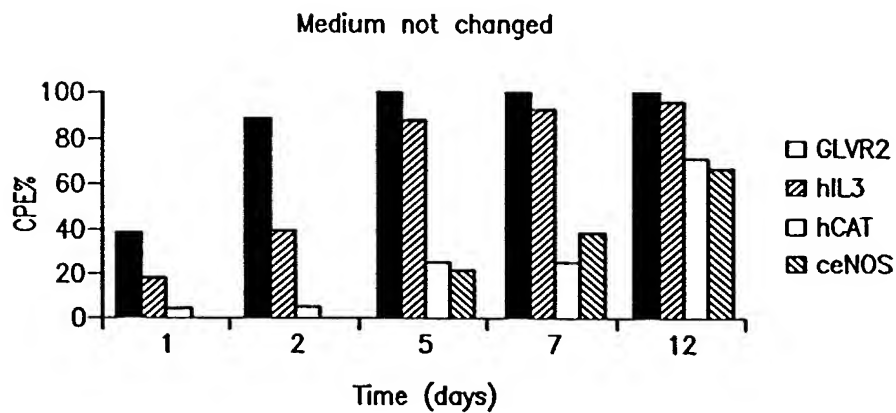


FIG. 38B

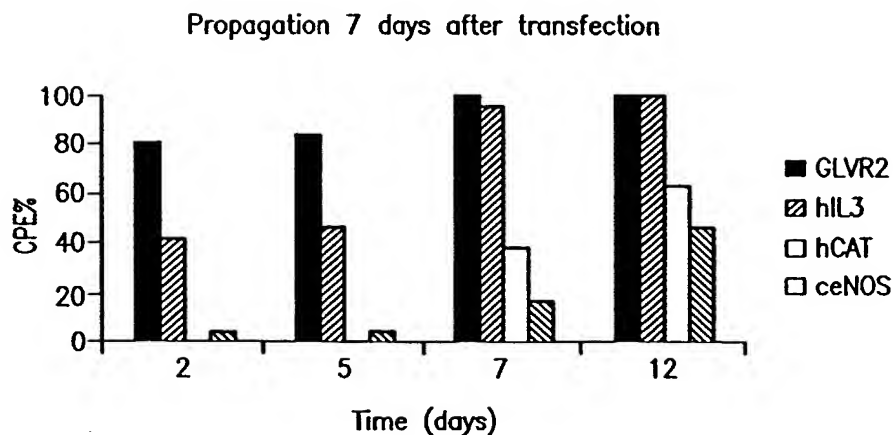


FIG. 38C

Cell titration experiment #1

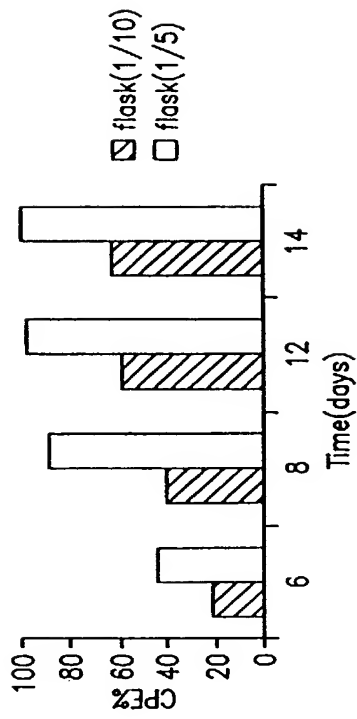


FIG. 39A

Cell titration experiment #2

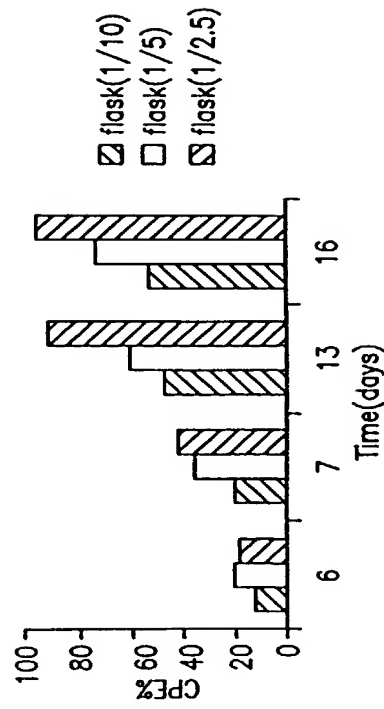


FIG. 39B

Cell titration experiment #3

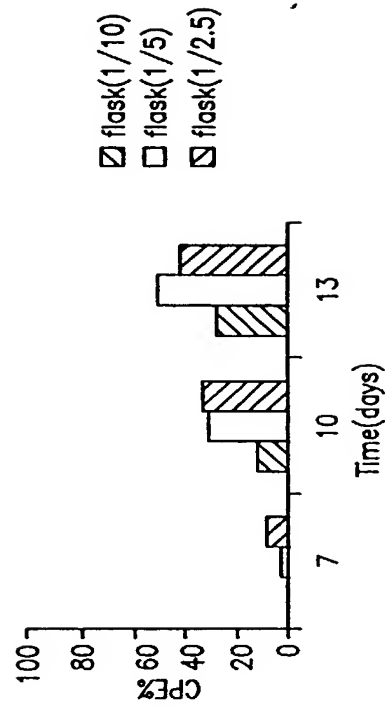


FIG. 39C



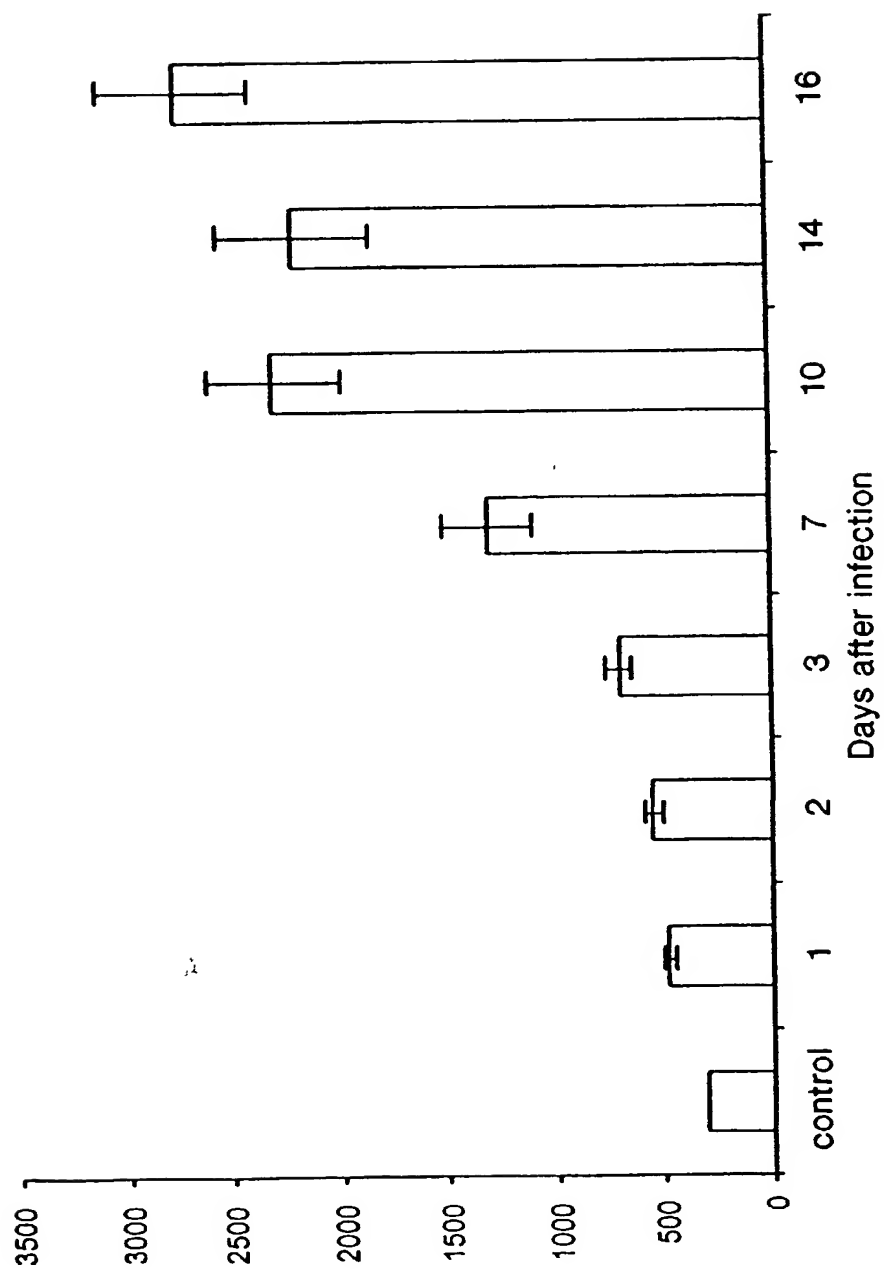


FIG. 40

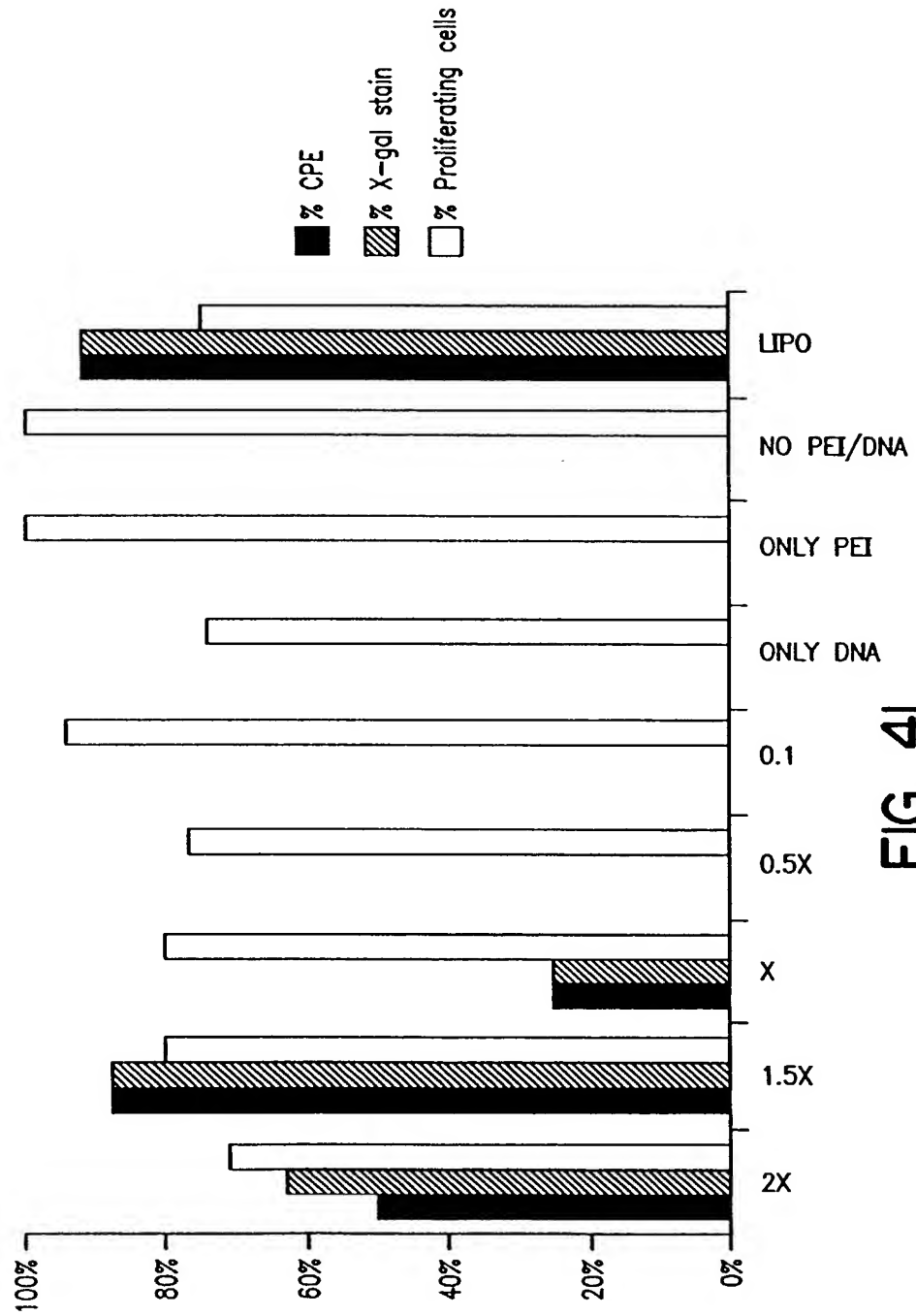
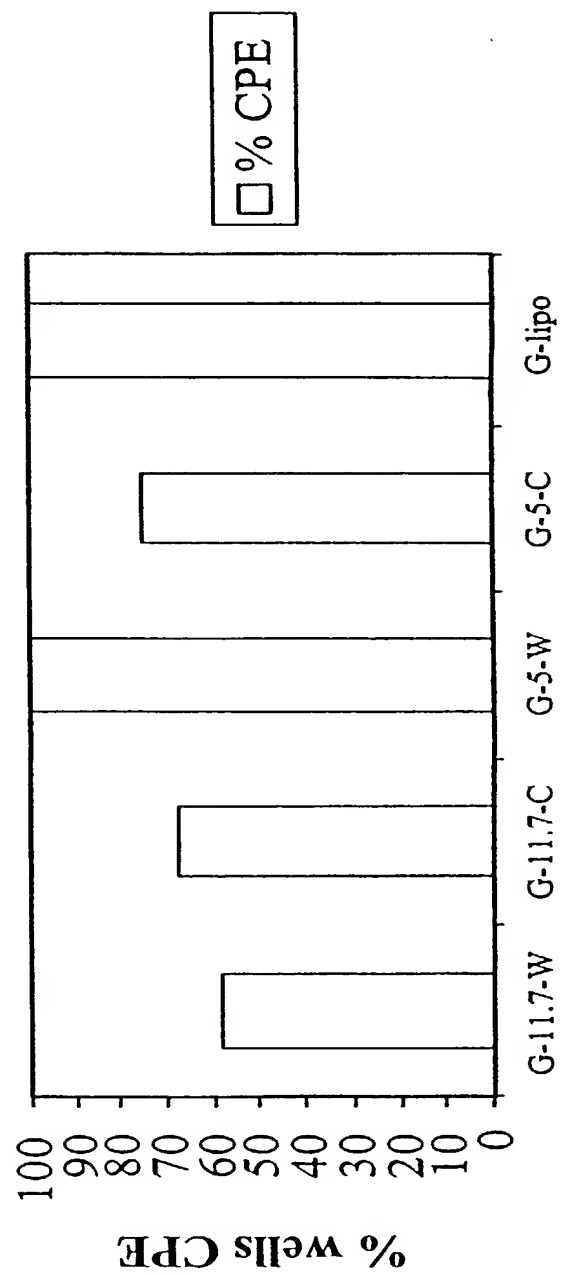


FIG. 4I

[illegible]

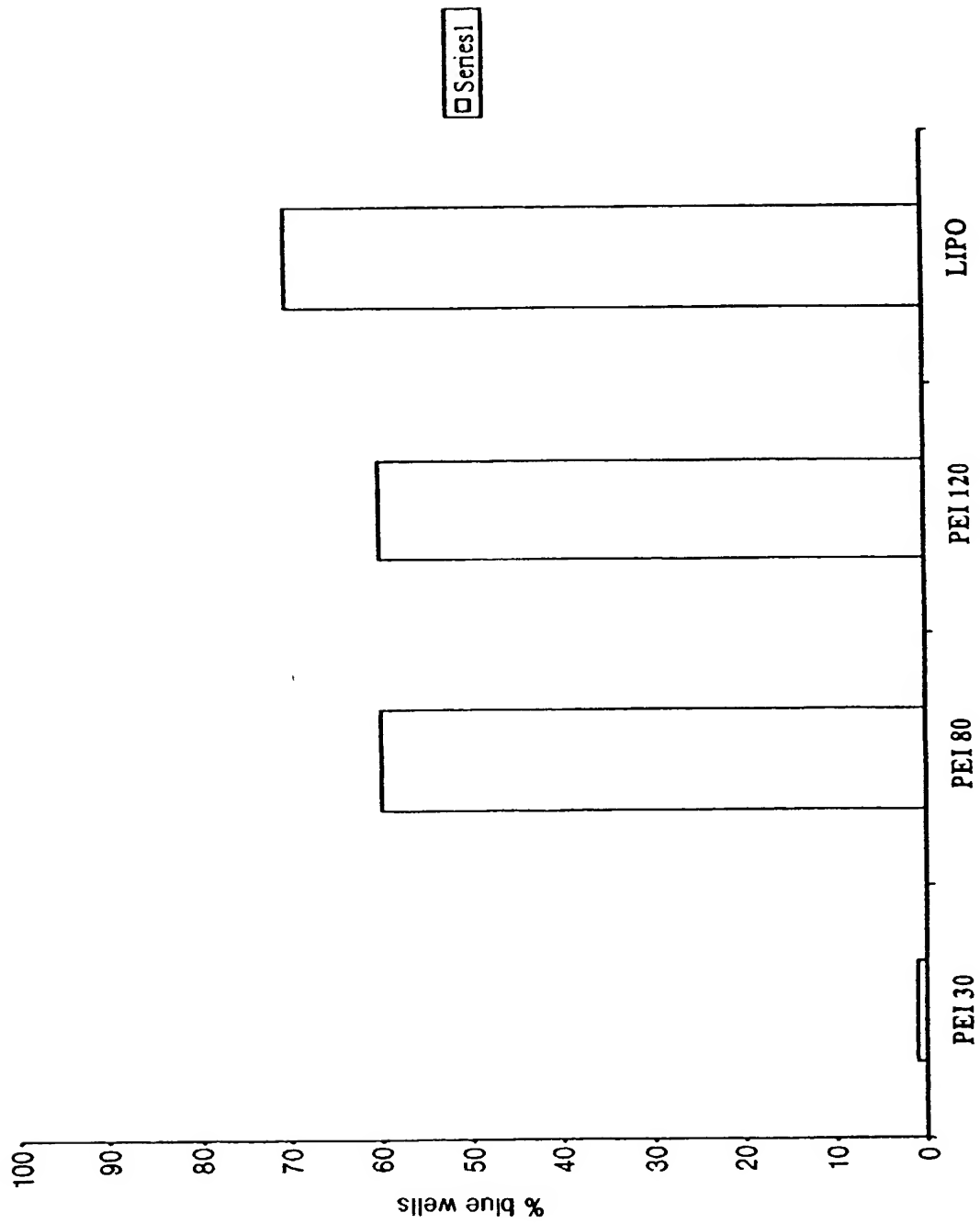


FIG. 43

FIG. 43

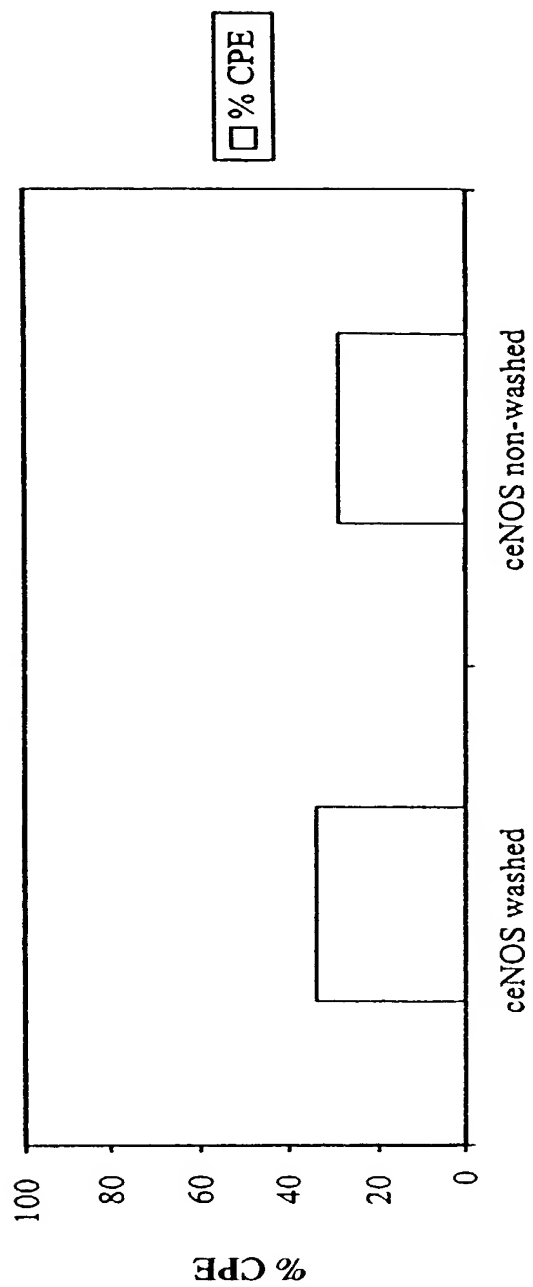


FIG. 44

FIG. 44 is a bar chart showing the percentage of CPE (Cytotoxic Plaque Endpoints) for two conditions: 'ceNOS washed' and 'ceNOS non-washed'. The y-axis is labeled '% CPE' and ranges from 0 to 100 in increments of 20. The x-axis has two categories: 'ceNOS washed' and 'ceNOS non-washed'. A legend indicates that the bars represent '% CPE'. The 'ceNOS washed' bar reaches approximately 30% on the y-axis, while the 'ceNOS non-washed' bar reaches approximately 25%.

Figure 45

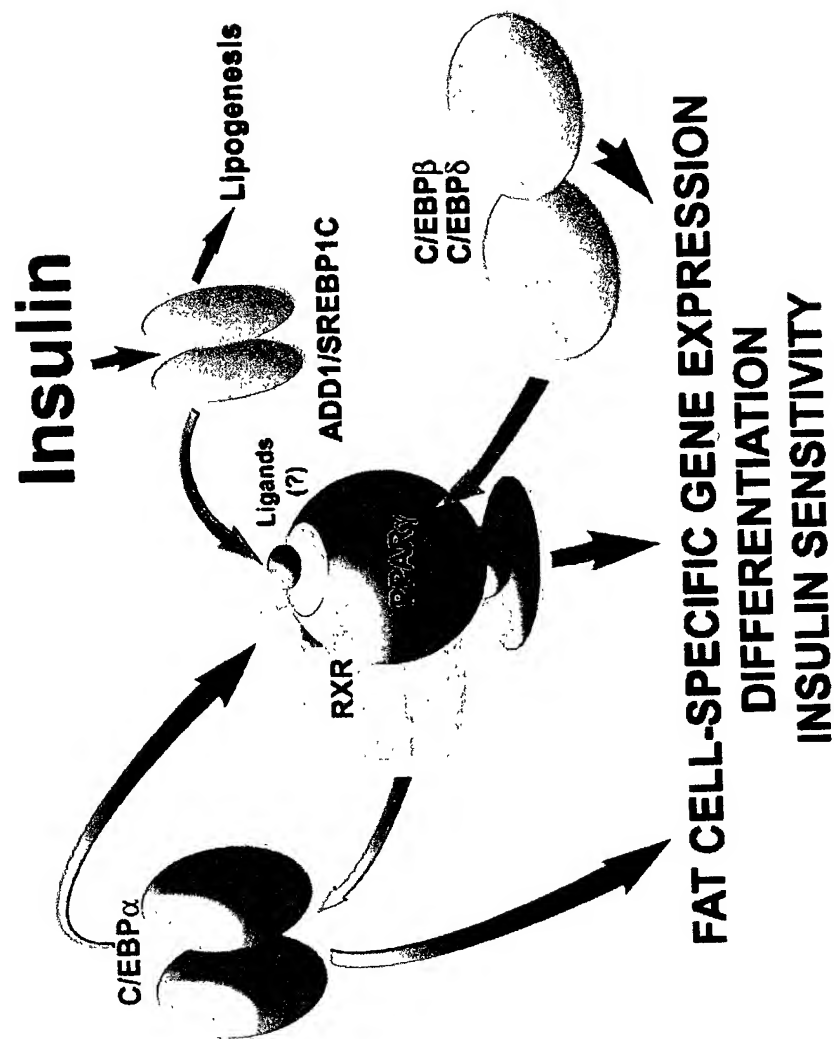


Figure 46

Transduction of hCAR<sup>-</sup> cells with Ad5

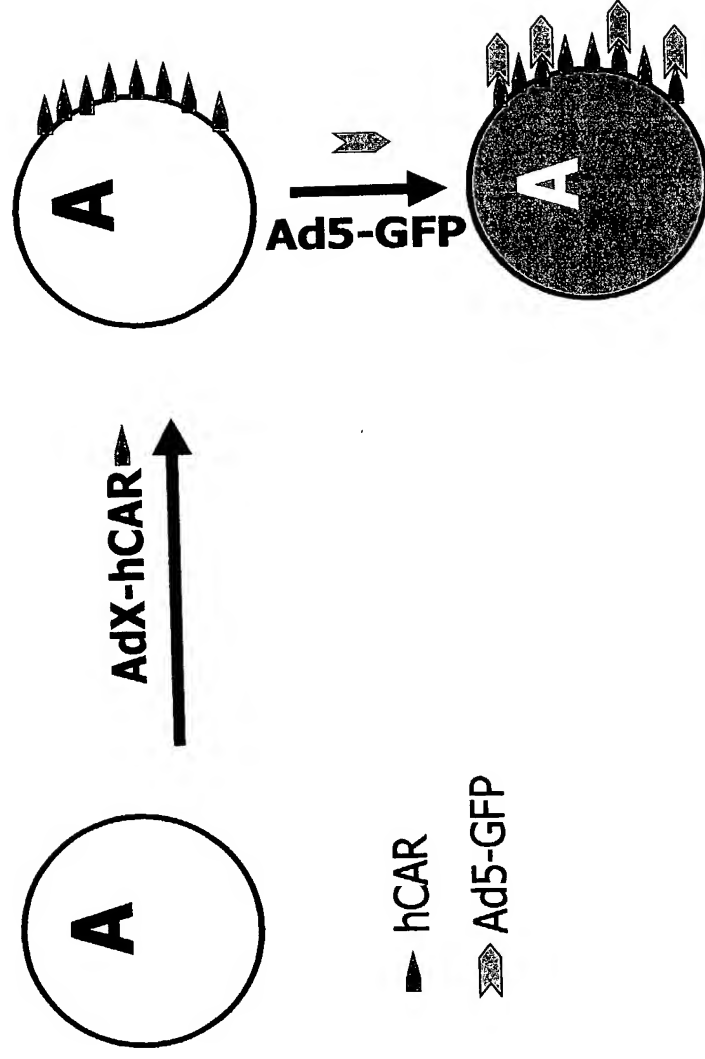
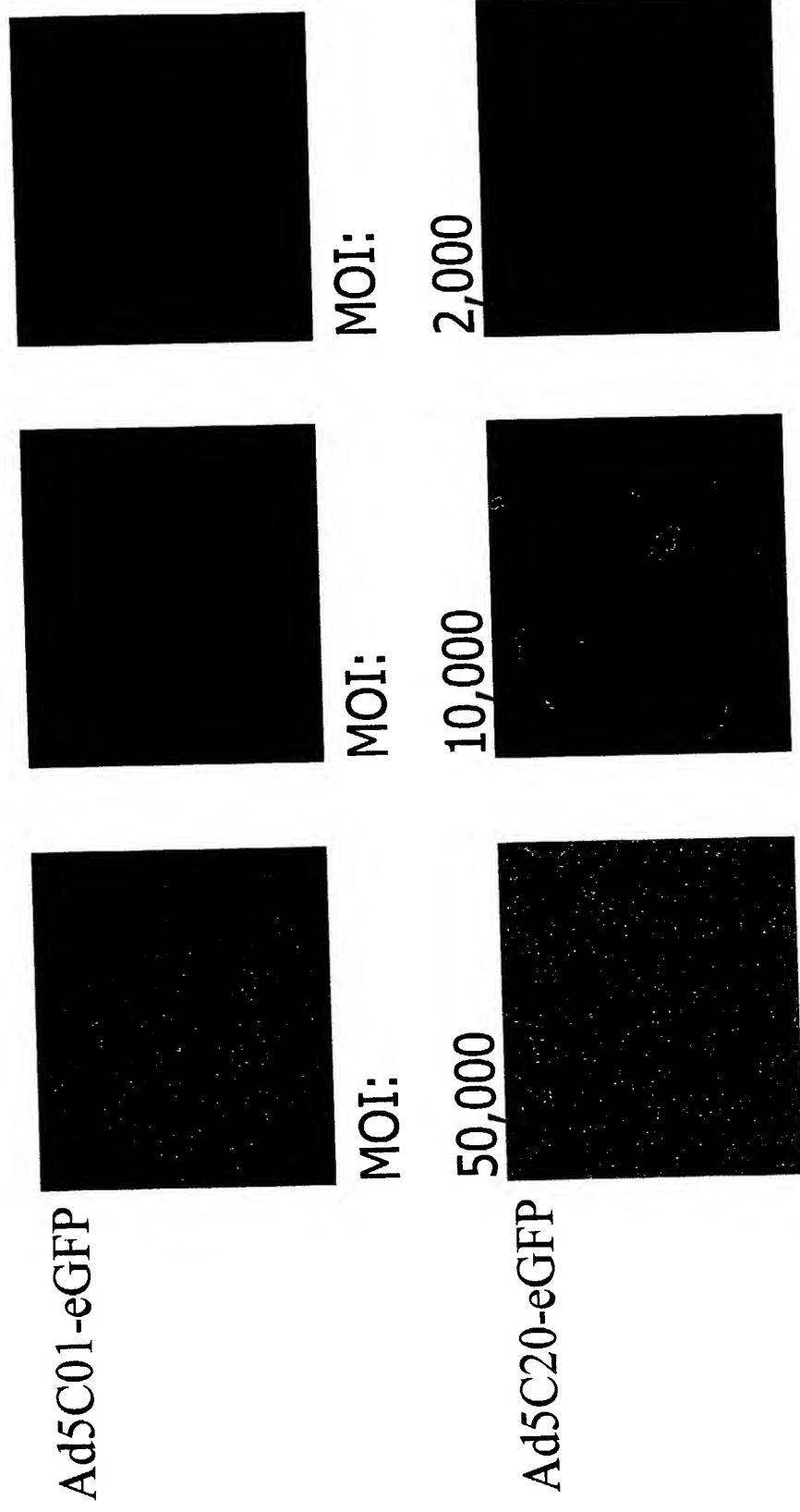


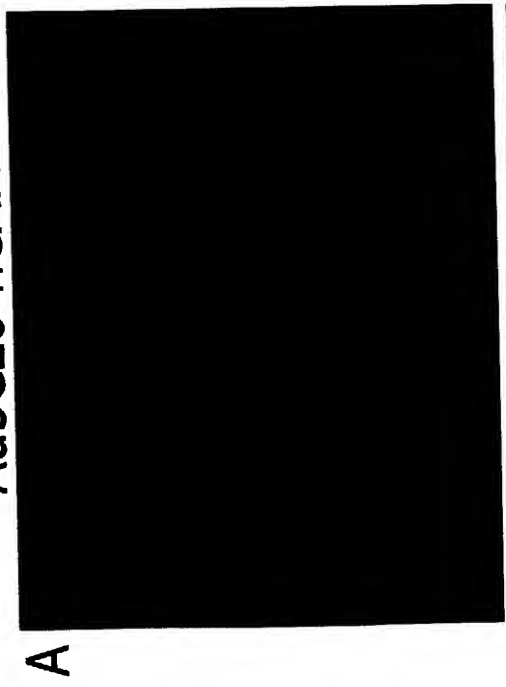
Figure 47

# Infection of human primary pre-adipocytes using Ad5C01 and Ad5C20 fiber-modified viruses



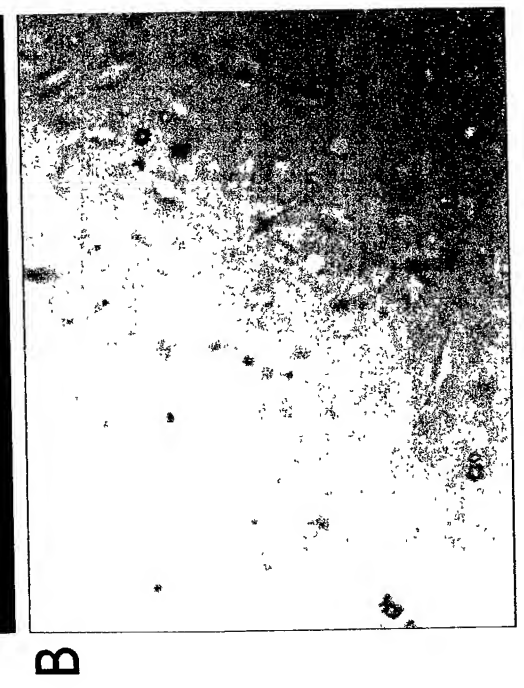
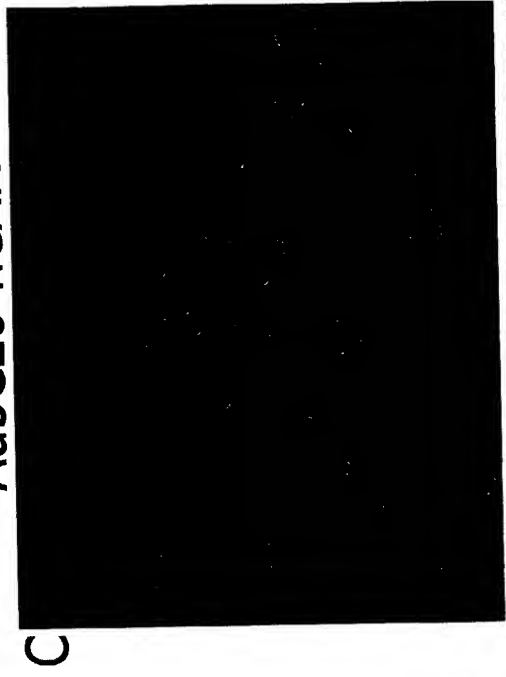


Ad5C01-Empty  
+  
Ad5C20-hCAR



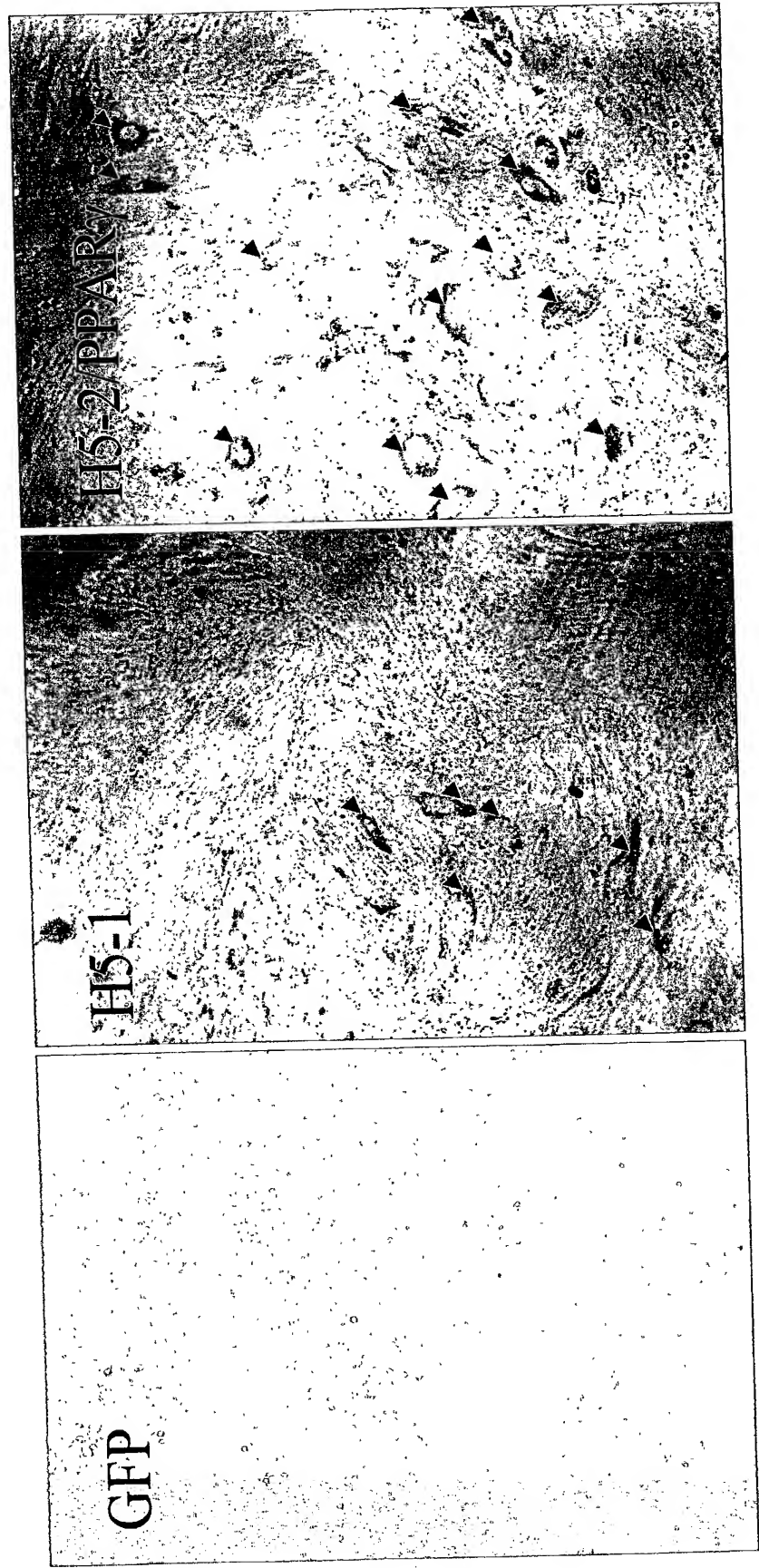
Ad5C01-PPAR $\gamma$   
+  
Ad5C20-hCAR

Figure 48



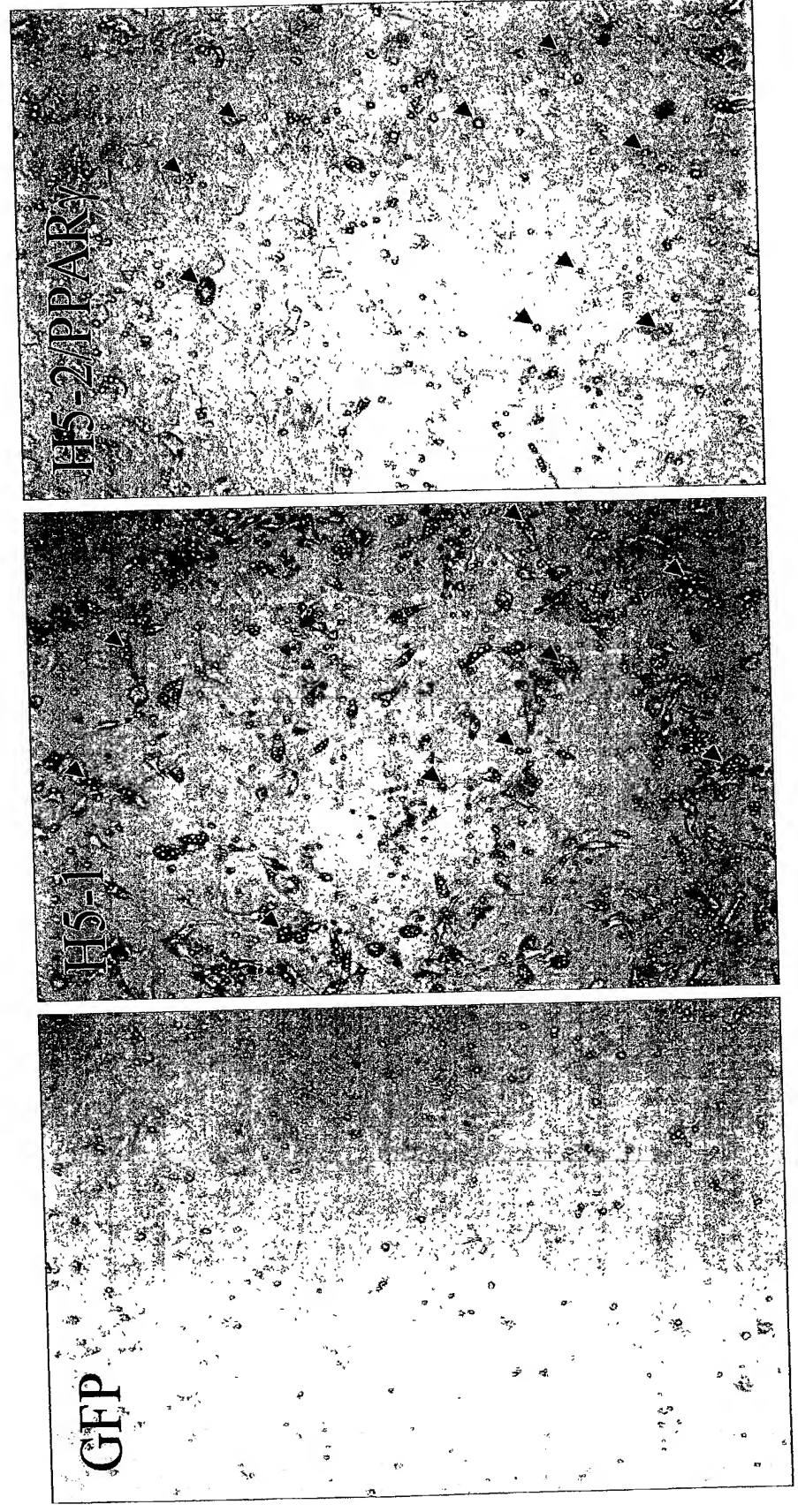
Adipocyte differentiation  
Primary human mesenchymal stem cells

Figure 49



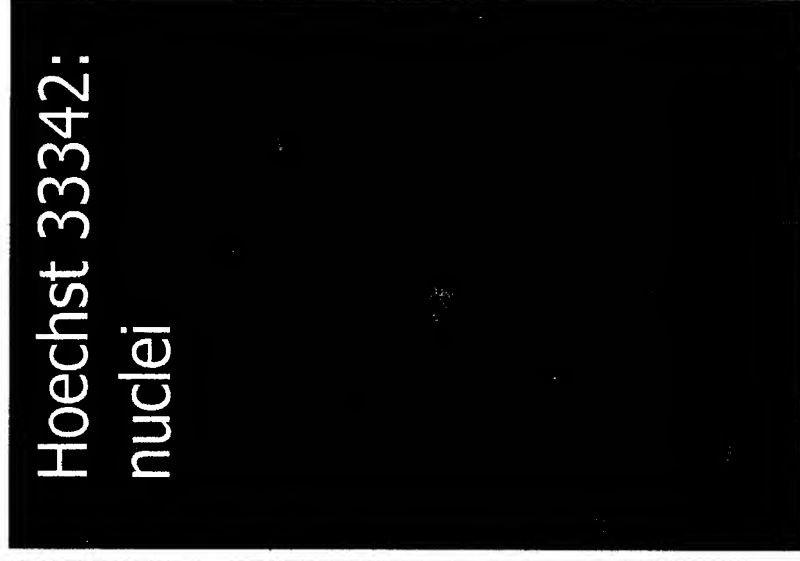
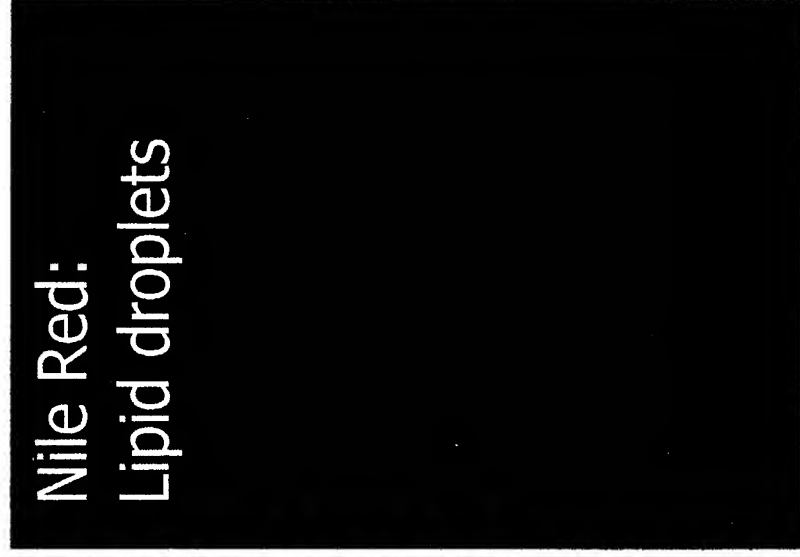
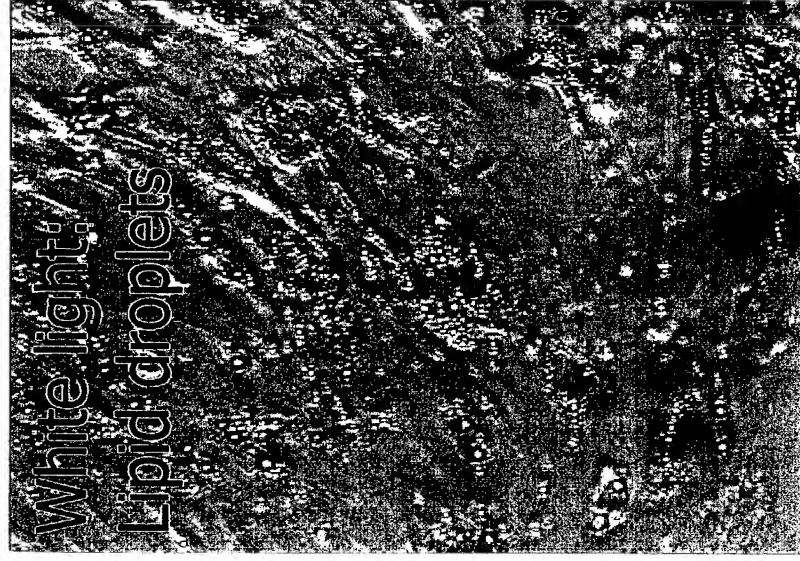
Adipocyte differentiation  
Mouse mesenchymal stem cell line  
C3H10T1/2

Figure 50



H5-24: adenovirally mediated expression  
of CIDEB does not induce any cell death

Figure 51



# FIGURE 52 H5-1 DNA sequence (SEQ ID NO:12)

```

1  GCCCACGCGT  CCGGTTTTCT  ACTTTGCCAC  AGATTATCTT  GTACAGCCTT  TTATGGACCA
61  ATTAGCATTC  CATCAATTTT  ATATCTAGCA  TATTTGCGGT  TAGAATCCCA  TGGATGTTTC
121  TTCTTTGACT  ATAACAAAAT  CTGGGGAGGA  CAAAGGTGAT  TTTCTGTGT  CCACATCTAA
181  CAAAGTCAAG  ATTCCCGGCT  GGACTTTTGC  AGCTTCCTTC  CAAGTCTTCC  TGACCACCTT
241  GCACTATTGG  ACTTTGGAAG  GAGGTGCCTA  TAGAAAACGA  TTTTGAACAT  ACTTCATCGC
301  AGTGGACTGT  GTCCCTCGGT  GCAGAAACTA  CCAGATTTGA  GGGACGAGGT  CAAGGAGATA
361  TGATAGGCCC  GGAAGTTGCT  GTGCCCCATC  AGCAGCTTGA  CGCGTGGTCA  CAGGACGATT
421  TCACTGACAC  TCGGAACCTC  CAGGACTACC  GTTACCAAGA  GGTTAGGTGA  AGTGGTTTAA
481  ACCAAACGGA  ACTCTTCATC  TTAAACTACA  CGTTGAAAAT  CAACCCAATA  ATTCTGTATT
541  AACTGAATTC  TGAACCTTTC  AGGAGGTACT  GTGAGGAAGA  GCAGGCACCA  GCAGCAGAAI
601  GGGGAATGGA  GAGGTGGGCA  GGGGTTCCAG  CTTCCCTTTG  ATTTTTTGCT  GCAGACTCAT
661  CCTTTTAAA  TGAGACTTGT  TTTCCCCTCT  CTTTGAGTCA  AGTCAAATAT  GTAGATTGCC
721  TTTGGCAATT  CTTCTTCTCA  AGCACTGACA  CTCATTACCG  TCTGTGATTG  CCATTTCTTC
781  CCAAGGCCAG  TCTGAACCTG  AGGTTGCTTT  ATCCTAAAAG  TTTTAACCTC  AGGTTCCAAA
841  TTCAGTAAAT  TTTGGAAACA  GTACAGCTAT  TTCTCATCAA  TTCTCTATCA  TGTTGAAGTC
901  AAATTTGGAT  TTTCCACCAA  ATTCTGAATT  TGTAGACATA  CTTGTACGCT  CACTTGCCCC
961  AGATGCCTCC  TCTGTCTCTA  TTCTTCTCTC  CCACACAAGC  AGTCTTTTTT  TACAGCCAGT
1021  AAGGCAGCTC  TGTCGTGGTA  GCAGATGGTC  CCATTATTCT  AGGGTCTTAC  TCTTTGTATG
1081  ATGAAAAGAA  TGTGTTATGA  ATCGGTGCTG  TCAGCCCTGC  TGTCAGACCT  TCTTCCACAG
1141  CAAATGAGAT  GTATGCCCAA  AGACGGTAGA  ATTAAAGAAG  AGTAAAATGG  CTGTTGAAGC
1201  AAAAAAAAAA  AAAAA

```

## FIGURE 53

H5-24 DNA sequence (SEQ ID NO:14)

```

1  GTCGACCCAC GCGTCCGCGC CTGCAGAAGG TTGACTGCGT GGTAGGGGGC CCAGAGCAAG
61  CCGAAGGCCA GCACGATGGC GCTCACCAGC CGGCCACCC GCGCCCCGTG CCGCCCGGAG
121  CCCCAGCGGG CGCCCCGAG CCGTGCCAGC GTCACGCTGT AGCAGCCGAG CATCAGCCCCG
181  AAAGGAAGCA CGAAAGCGGT GGCGGTAGAC GGCGGCCGGG ACGGCGAGCA ACAGGGCGGC
241  CAGCCAGACC GCCAGCAGCA GGCGGCCGGG CAGGGCCGGG CTGCGCAGCC GAGGCGCCAG
301  GAAGGGGCGG GTGACTGCGA GGCAGCGCTG CAGGCTGAGC AGGCCGGTGA GCAGCACGCT
361  GGCGTACATG CTGAGCGCGC ACACGTAGTA CACCGCCTTG CAGCCCGCCT GGCCACGCGG
421  CCAGGCCTGC CGGGTCAGGA AGGCCACAAA GAGCGGCGTG AGCAGCAGCA CCGCGCCGTC
481  GGCCAGCGCC AGGTGCAGCA CAAGCGTGGC CGCCAGCGGT CGCCCCCGTG CAGGCCGCCA
541  GCCCGCCAAG CTCCACACCA CGAAGCCGTT GCCAGGCAGC CCCAGCAGCG CCGCCAGCAG
601  CAGGAAGGCT GTGCCTGTGG CCCGCGAAGT CTTCAGCTC AGCAGTGTCT CGTTCCCTGG
661  GGGACGGTAG CAGACCGACA TCCTTCTGGG CCTACAGGAC ACAGAAAAAA AGTGGGGAAG
721  CTGGGGGACC CCTACAAGGA TCCTTGGCAG GAAAGCAGGG ATTGTGTTCA TTTGAGGGTT
781  TCACTGTCTG TGAGAGTCTC AGCTTCCATG CAACTGTCCA TCACGGCTGC AACTGAAATC
841  AGAGCTGGGA CACAGCGCAC CAGAAGCTAA AGTCTTGATG CCATCAAAGG ACATCCCTGC
901  CCCATTCACT TCTCTGTAC GTCCACTAAT CGGCAAAAGG AGAAAAGTGA GAGAAGATGA
961  CCTAAGTGTG ACTGCAGCAG GCAGCTCTGG AAAATGAAGC CAGAGCAGTG AGCCAGCCCC
1021  TCCTCCGACC AAGGAGGAAG GAAAGAGCAG CCCCAGCACA GGAGAGAACC ACCCAGCCCCA
1081  GAAGTTCCAG GGAAGGAAGT CTCCGGTCCA CCATGGAGTA CCTCTCAGCT CTGAACCCCA
1141  GTGACTTACT CAGGTCAGTA TCTAATATAA GCTCGGAGTT TGGACGGAGG GTCTGGACCT
1201  CAGCTCCACC ACCCCAGCGA CCTTTCCGTG TCTGTGATCA CAAGCGGACC ATCCGGAAAG
1261  GCCTGACAGC TGCCACCCGC CAGGAGCTGC TAGCCAAAGC ATTGGAGACC CTAAGTCTGA
1321  ATGGAGTGCT AACCTGGTG CTAGAGGAGG ATGGAAGTGC AGTGGACAGT GAGGACTTCT
1381  TCCAGCTGCT GGAGGATGAC ACGTGCCTGA TGGTGTGCA GTCTGGTCAG AGCTGGAGCC
1441  CTACAAGGAG TGGAGTGCTG TCATATGGCC TGGGACGGGA GAGGCCAAG CACAGCAAGG
1501  ACATCGCCCG ATTCACCTTT GACGTGTACA AGCAAAACCC TCGAGACCTC TTTGGCAGCC
1561  TGAATGTCAA AGCCACATTC TACGGGCTCT ACTCTATGAG TTGTGACTTT CAAGGACTTG
1621  GCCCAAAGAA AGTACTCAGG GAGCTCCTTC GTTGGACCTC CAACTGCTG CAAGGCCTGG
1681  GCCATATGTT GCTGGGAATT TCCTCCACCC TTCGTCATGC AGTGGAGGGG GCTGAGCAGT
1741  GGCAGCAGAA GGGCCGCCTC CATTCCTACT AAGGGGCTCT GAGCTTCTGC CCCAGAATC
1801  ATTCCAACCG ACCCACTGCA AAGACTATGA CAGCATCAAA TTTCAGGACC TGCAGACAGT
1861  AAGGCTAGA TAACCCACCC AATTTCCCCA CTGTCCTCTG ATCCCCTCGT GACAGAACCT
1921  TTCAGCATAA CGCCTCACAT CCCAAGTCTA TACCTTACC TGAAGAATGC TGTCTTTTC
1981  TAGCCACCTT TCTAGCCTCC CACTTGCCCT GAAAGGCCAA GATCAAGATG TCCCCAGGC
2041  ATCTTGATCC CAGCCTGACT GCTGCTACAT CTAATCCCCT ACCAATGCCT CCTGTCCCTA
2101  AACTCCCCAG CATACTGATG ACAGCCCTCT CTGACTTTAC CTTGAGATCT GTCTTCATAC
2161  CCTTCCCCTC AAATAACAA AACATTTTCC AATAAAAATA TCAAATATTT AAAAAAAAAA
2221  AAAAAAGGG CGGCCGC

```

## FIGURE 54

H5-24 ORF4 Amino Acid sequence (SEQ ID NO: 71)

MEYLSALNPSDLLRSVSNISSEFGRRVWTSAPPPQRPFRVCDHKRTIRKGLTAAT  
RQELLAKALETLLLNGVLTTLVLEEDGTAVDSEDDFFQLLEDDTCLMVLQSGQSW  
PTRSGVLSYGLGRERP KHSKDIARFTFDVYKQNPRDLFGSLNVKATFYGLYSMS  
CDFQGLGPKKVLRELLRWTSTLLQGLGHMLLGISSTLRHAVEGAEQWQKGR  
LSY

## FIGURE 55

H5-24 Segment 1 of BLTR2 DNA sequence (SEQ ID NO: 15)

```
18 CGC CTGCAGAAGG TTGACTGCGT GGTAGGGGGC CCAGAGCAAG
61 CCGAAGGCAA GCACGATGGC GCTCACCAGC CGGCCCACCC GCGCCCCGTG CCGCCCGGAG
121 CCCCAGCGGG CGCCCCGCAG CCGTGCCAGC GTCACGCTGT AGCAGCCGAG CATCAGCCCG
181 AAAGGAAGCA CGAAAGCGGT 200
```



## FIGURE 56

H5-24 Segment 2 DNA sequence (SEQ ID NO: 16)

```
198 GGT GGC GGCGGTAGAC GGCGGCCGGG ACGGCGAGCA ACAGGGCGGC
241 CAGCCAGACC GCCAGCAGCA GGCGGCCGGG CAGGGCCGGG CTGCGCAGCC GAGGCGCCAG
301 GAAGGGGCGG GTGACTGCGA GGCAGCGCTG CAGGCTGAGC AGGCCGGTGA GCAGCACGCT
361 GGCGTACATG CTGAGCGCGC ACACGTAGTA CACCGCCTTG CAGCCCGCCT GGCCAGCGG
421 CCAGGCCTGC CGGGTCAGGA AGGCCACAAA GAGCGGCGTG AGCAGCAGCA CCGCGCCGTC
481 GGCCAGCGCC AGGTGCAGCA CAAGCGTGGC CGCCAGCGGT CGCCCCCGTG CAGGCCGCCA
541 GCCCGCCAAG CTCCACACCA CGAAGCCGTT GCCAGGCAGC CCCAGCAGCG CCGCCAGCAG
601 CAGGAAGGCT GTGCCTGTGG CCCGCGAAGT CTTCCAGCTC AGCAGTGTCT CGTTCCCTGG
661 GGGACGGTAG CAGACCGACA TCC'TTCTGGG CCTACAGG 698
```

## FIGURE 57

### DNA Sequence Comparison of H5-24 Segment 1 (SEQ ID NO: 15) with BLTR2

#### Antisense DNA sequence

SEQ ID NO:15	18	cgcctgcagaagggttgactgcgtggtagggggcccagagcaagccaaggcaagcacgat	77
BLTR2	2455	cgcctgcagaagggttgactgcgtggtagggggcccagagcaagccaaggcaagcacgat	2396
SEQ ID NO:15	78	ggcgctcaccagccggccccacccgcgccccgtgccgcccggagccccagcggcgccccg	137
BLTR2	2395	ggcgctcaccagccggccccacccgcgccccgtgccgcccggagccccagcggcgccccg	2336
SEQ ID NO:15	138	cagccgtgccagcgtcacgctgtagcagccgagcatcagcccgaagggaagcacgaaagc	197
BLTR2	2335	cagccgtgccagcgtcacgctgtagcagccgagcatcagcccgaagggaagcacgaaagc	2276
SEQ ID NO:15	198	ggt	200
BLTR2	2275	ggt	2273

## FIGURE 58

### DNA Sequence Comparison of H5-24 Segment 2 (SEQ ID NO: 16) with BLTR2

#### Antisense DNA sequence

SEQ ID NO:16	198	ggtggcggtagacggcgccgggacggcgagcaacagggcgccagccagaccgccagca	257
BLTR2	2195	ggtggcggtagacggcgccgggacggcgagcaacagggcgccagccagaccgccagca	2136
SEQ ID NO:16	258	gcaggcgggcgccagggcgccggctgcgagccgagggcgccaggaagggcggggtgactg	317
BLTR2	2135	gcaggcgggcgccagggcgccggctgcgagccgagggcgccaggaagggcggggtgactg	2076
SEQ ID NO:16	318	cgaggcagcgctgcaggctgagcaggccggtgagcagcacgctggcgtagatgctgagcg	377
BLTR2	2075	cgaggcagcgctgcaggctgagcaggccggtgagcagcacgctggcgtagatgctgagcg	2016
SEQ ID NO:16	378	cgcacacgtagtacaccgccttgcagcccgccctggcccagcgccagggcctgcccgggtca	437
BLTR2	2015	cgcacacgtagtacaccgccttgcagcccgccctggcccagcgccagggcctgcccgggtca	1956
SEQ ID NO:16	438	ggaaggccacaaagagcgggcgtgagcagcagcaccgcgccgtcgccagcgccaggtgca	497
BLTR2	1955	ggaaggccacaaagagcgggcgtgagcagcagcaccgcgccgtcgccagcgccaggtgca	1896
SEQ ID NO:16	498	gcacaagcgtggccgccagcggtcgcccccggtgcaggccgccagcccgccaagctccaca	557
BLTR2	1895	gcacaagcgtggccgccagcggtcgcccccggtgcaggccgccagcccgccaagctccaca	1836
SEQ ID NO:16	558	ccacgaagccggttgccaggcagccccagcagcgccgccagcagcaggaaggctgtgcctg	617
BLTR2	1835	ccacgaagccggttgccaggcagccccagcagcgccgccagcagcaggaaggctgtgcctg	1776
SEQ ID NO:16	618	tggcccgcggaagtcttccagctcagcagtgctctcgttccctgggggacggtagcagaccg	677
BLTR2	1775	tggcccgcggaagtcttccagctcagcagtgctctcgttccctgggggacggtagcagaccg	1716
SEQ ID NO:16	678	acatccttctgggcctacagg	698
BLTR2	1715	acatccttctgggcctacagg	1695